# Rat social touch: a behavioral and neurophysiological study

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# 1 Introduction

## 1.1 Whisking

Whiskers or vibrissae are stiff facial hairs used for tactile perception. Although they are present in nearly all mammals (Muchlinski, 2010), active movement of the vibrissae as a sensory sampling strategy ('whisking') is developed only in some orders. These include rodents (e.g. rats (Vincent, 1912) and mice (Jin et al., 2004)), insectivores[1] (e.g. Etruscan shrews; (Brecht et al., 2011)), and opossums (Mitchinson et al., 2011), which are not only a different order, but a different infraclass (marsupials, as opposed to placental animals). This wide prevalence shows the effectivity of whisking as a sensory strategy. In rodents, it has received particularly large attention, both due to its importance in their behavior (Ahl, 1986) and to the role of mice and rats as model organisms. Rats are nocturnal and spend much of their live in nests and burrows, and, as a consequence, they see very poorly, which is particularly true of albino rats, which reach acuities of only 0.5 cycles per degree (Prusky et al., 2002), or 1/100 of the optimal human performance. In contrast, a highly developed sense of touch provides substantial benefits in the environment preferred by rats.

Although immobile vibrissae can also be very useful in increasing sensitivity and tactile sampling area, whisking provides further improvements in sensory performance. In rats and mice, whisking comprises a regular sweeping movement of the whiskers along the antero-posterior axis of the animal. The frequency of this motion is typically reported to be in the range of 6-12 Hz for rats. Thus, in the earliest quantification of whisking frequencies, Welker (1964) observed predominantly frequencies of 7-9 Hz. Later, Carvell and Simons (1990) observed a main frequency of 8 Hz and Sachdev et al. (2001) reported whisking to occur at 6-12 Hz. There are, however, reports of whisking at higher frequencies as well, e.g., Berg and Kleinfeld (2003) found typical exploratory whisking to be in the range of 5-15 Hz, and additionally report a rarer 'foveal whisking' with small amplitudes at 15-25 Hz. These high frequencies are, however, rarely reported in rats, and the term 'foveal whisking' has also been used for whisking with 9 Hz by Semba and Komisaruk (1984), who found exploratory whisking to occur at 7 Hz.

There are numerous studies on the active use of whiskers for the discrimination of textures (Carvell and Simons, 1990; Morita et al., 2011), vibrations (Adibi et al., 2012), and antero-posterior location (Knutsen et al., 2006), and some of these show remarkable sensitivities. For example, Knutsen et al. (2006) observed acuities of 0.24 mm or 1° radial distance for contact pole localization, and Morita et al. (2011) showed discrimination between sandpapers of 100 and 82 µm grit size. The neural basis of these discrimination abilities had also been investigated, partly with awake freely-moving animals (Krupa et al., 2004; von Heimendahl et al., 2007; Jadhav et al., 2009).

While whisking behaviors have been formerly mostly investigated in rats, the availability of optogenetic manipulation in mice has led to a recent increase of studies on the mouse

[1] The order 'insectivora' is still commonly used, but is not monophyletic, and is thus falling into disuse amongst zoologists. However, it is still widely used outside of biological systematics.

whisker system. Thus, it was shown that mice can discriminate anteroposterior (O'Connor et al., 2010b) and radial (Pammer et al., 2013) locations of contact poles with a precision of <1 mm, and there have been detailed studies of neuronal activity associated with tactile discrimination (O'Connor et al., 2010a; Xu et al., 2012).

There is, however, a lack of studies which show how rodents use their whisker to contact objects of more complex shape than apertures and poles, in particular when allowed to investigate them freely, and how such shapes are represented on the neuronal level. In one study, Grant et al. (2012) could show that after orienting towards an unexpected object, close contact is established, and there is a 'dabbing' of microvibrissae (Brecht et al., 1997) against the object. There are, however, as yet no studies on the integration of signals from micro- and macrovibrissae on both behavioral and neuronal levels, although this is presumably very important for the detection and tactile categorization of objects.

## 1.2 Rat behavior and social whisking

Whiskers have also been shown to play an important role in rat social behavior. Generally, rats are social animals which live in groups and display an array of social behaviors, which mostly fall into the categories of parental (Wiesner and Sheard, 1933), mating (Erskine, 1989), play (Panksepp, 1981), and hierarchy-related agonistic behaviors (Blanchard et al., 1975; also see Fig. 1.1), as well as social transmission of signals including those related to food (Galef and Kennett, 1987), danger (Blanchard et al., 1991), and stress (Valenta and Rigby, 1968). The use of whiskers in hierarchy-related agonistic behaviors is particularly well-documented (Blanchard et al., 1977b; Wolfe et al., 2011).

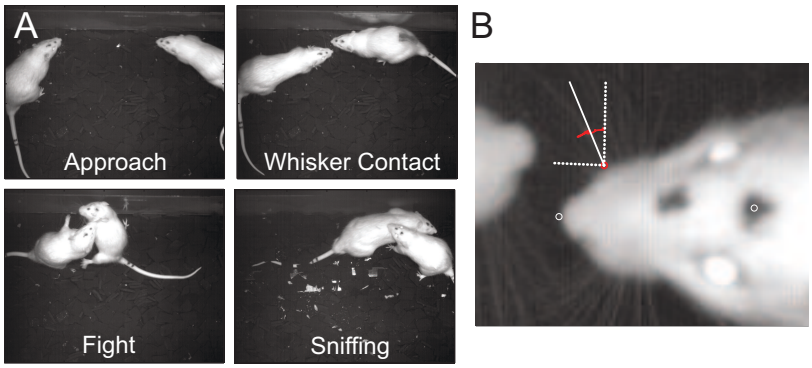



Figure 1.1: Example of whisker use in free interactions and different rat social behaviors. A, Rats approach and contact each other with their whiskers (top). Interactions of male rats then often continue with either fights (bottom left) or sniffing (bottom right). B, Still figure from an unrestrained social interaction, showing overlap of the rats' whisker arrays. White solid line indicates the position of the tracked whisker in this particular frame. Red trace indicates whisker tag positions in previous frames. White dashed lines mark the coordinate system, defined by the rat midline and a line perpendicular to it. Modified with permission from Wolfe et al. (2011)

In a behavior called 'boxing', rats stand opposite to each other on their hindlimbs and keep their whiskers protracted towards the other rat's face, presumably as a way to avoid being bitten (Blanchard et al., 1977a). Rats also exhibit strong whisking during anogenital sniffing, and, importantly, during social facial interactions (Wolfe et al., 2011). These are non-aggressive encounters, in which rats approach each other frontally, and engage in an interaction during which typically both rats move their whiskers, and which often comprises touch of the heads at the or close to the nose tip (Fig. 1.2). In the gap paradigm, as employed in this study, this behavior is initiated repeatedly and voluntarily (Wolfe et al., 2011). Importantly, this behavior is both initiated and sustained without any behavioral training or reward. While anogenital sniffing is very prominent when two rats begin to interact freely, facial interactions increase in frequency over time and eventually become the dominant type of social interactions (Wolfe et al., 2011). When rats are allowed to see, hear, and smell each other, but are barred from touching each other, there is a rebound effect, indicating the ethological significance of this behavior (Wolfe et al., 2011). This is also hinted at by the finding that ultrasound vocalizations occur at a higher frequency during social facial touch (F. Mielke and R. Rao, personal communication).

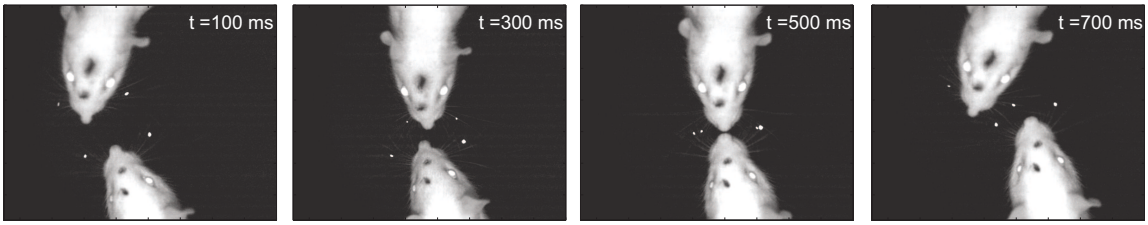



Figure 1.2: Sequence of stills at different stages of a social facial interaction. At nose contact, rats assume a stereotyped alignment with the head midlines being in one line. Most interactions are longer than this example. The whisker tags are clearly visible as white dots. Reproduced with permission from Wolfe et al. (2011).

The precise function(s) of social facial touch is/are, however, not known. By sampling their conspecifics with their whiskers, rats could obtain information on the size and possibly sex of the partner. The whisking movements of the partner could also carry relevant information. Finally, non-tactile functions are possible, in particular the sampling of non-volatile odors from the cheek glands (Kannan and Archunan, 2001; Kiyokawa et al., 2004). The importance of olfactory cues is supported by the finding that for individual recognition odors are both necessary and sufficient (Gheusi et al., 1997).

The capability of rats to engage in complex social relationships with conspecifics is also highlighted by observations of general reciprocity in rats (Rutte and Taborsky, 2007). If female rats received help, they later helped other rats more often to obtain food, without

directly profiting from it themselves. Rats might even show empathy (Ben-Ami Bartal et al., 2011), as assessed by a paradigm in which rats can liberate conspecifics from restrainment. Although this interpretation has been called into question (Silberberg et al., 2013), even the alternative explanation offered by the authors requires an intrisic need for social interactions.

## 1.3 Barrel cortex and the cortical representation of touch

The neural representations of the whiskers (Fig. 1.3A) and the neuronal computations associated with the processing of tactile stimuli provided by them have been one of the central fields of study of sensory neuroscience, ever since Woolsey and Van der Loos (1970) described the topographical representation of the whisker pad in primary somatosensory cortex (S1). This representation is known as the barrel cortex (BC), and is comprised of a field of topographically organized cortical columns, where each represents one of the macrovibrissae in the five whisker rows and six to seven whisker columns (Fig. 1.3B; also see Brecht et al., 1997). These cortical columns, which are characterized by prominent cytochrome oxidase staining in layer 4 (L4), are separated by septal compartments with distinct functions. Thus, the whisker/barrel cortex system is 'quantized' into single whiskers and their corresponding cortical columns, and involves active sensory strategies. This makes it a particularly valuable system for the study of sensory processing and sensorimotor integration on both behavioral and neural level. The latter includes the trigeminal nuclei, where the whisker afferents are first processed, the thalamic relay stages, and, most importantly, the BC as the primary somatosensory area of the neocortex. In addition, studies on sensorimotor integration also encompass primary motor cortex (M1), as well as other cortical and subcortical areas involved in the generation of whisking patterns and their control.

The flow of sensory information to the BC, the computations performed within it, and the projections to and interactions with other areas have been subject to detailed study, and have been reviewed in depth elsewhere (Lübke and Feldmeyer, 2007; Diamond et al., 2008; Bosman et al., 2011; Feldmeyer et al., 2013). In a short and somewhat simplified view, summarized in Fig. 1.4, the information reaches the BC through three channels: the lemniscal, the extralemniscal, and the paralemniscal pathways. The lemniscal is the most canonical one, which leads through the principal trigeminal nucleus and the ventral posterior medial nucleus of the thalamus to BC, where it targets mostly L4 neurons in barrels, but also L5B and L6 (Fig. 1.3C, Fig. 1.4). This pathway provides fast inputs with small receptive fields (RFs). The extralemniscal and paralemniscal pathways both originate in the spinal trigeminal nucleus, although in different substructures, and both provide slower inputs with larger RFs. They are also subject to stronger influence by top-down modulation related to whisker movements. The paralemniscal pathway gets relayed in the posterior medial thalamic nucleus, and targets mostly L5A, as well as L2/3 and L1 (Fig. 1.3C, Fig. 1.4). The extralemniscal pathway has only weak projections to S1, and these specifically target septal compartments. Further

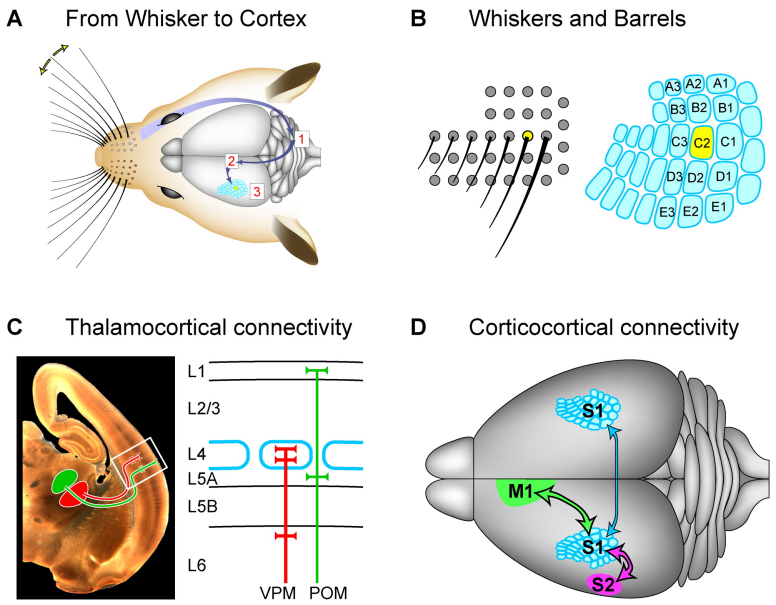



Figure 1.3: Overview over the whisker system of rats. A, Information from whisker touch is relayed through the brainstem (1) and the thalamus (2) to the barrel cortex (3). B, Whiskers are arranged in an orderly array, and there is a topographical mapping of whiskers onto barrels in the barrel cortex. C, The main two processing pathways are the lemniscal pathway (red) which routes information to the barrel cortex through the ventral posterior medial nucleus of the thalamus (VPM), and the paralemniscal pathway (green), leading through the posterior medial nucleus of the thalamus (POm). D, Within cortex, S1 is strongly and reciprocally connected to primary motor cortex (M1) and secondary somatosensory cortex (S2), as well as the contralateral barrel cortex. Reproduced with permission from Petersen (2007).

pathways exist, which are involved in multi-whisker processing, and preferentially target the septa. This diversity of input streams and compartments is an important limitation to the interpretation of extracellular recordings, which almost unavoidably pool barrel and septal units, and are insensitive to cortical microcircuitry, which might be spatially intermingled and still computationally separated.

Within BC, the information flowing through the lemniscal pathway is relayed by L4 neurons to neurons in L2/3, which in turn excite L5 neurons through their apical dendrites (Fig. 1.4). Partly already in L2/3, but most strongly in L5A, the lemniscal inputs then interact with paralemniscal inputs reaching the cortex at longer latencies, and carrying information regarding a broader set of whiskers. In addition to the vertical flow of information between layers, there are strong horizontal, intralaminar connections, which are mostly local in L4, but are connecting different barrels in L2/3 and L5B. These tactile information flows are constantly shaped by a multitude of top-down inputs from M1, secondary somatosensory cortex (S2), and secondary thalamic nuclei, amongst others. There are also inputs from the contralateral BC, which convey information related to the ipsilateral whisker field. This view of

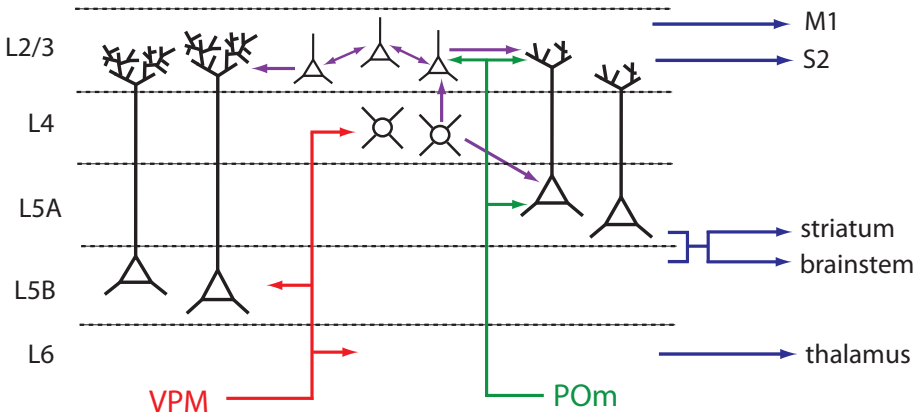



Figure 1.4: Schematic overview over the most important projections to and from, as well as connections within BC. Lemniscal inputs are marked in red, paralemniscal in green, projections to other areas in blue and connections within BC in violet. Thick-tufted and slender-tufted L5 neurons are more prominent in L5B and L5A, respectively, as shown here. However, cell type and layer do not map completely in this case. Layer thicknesses and cell sizes do not reflect actual anatomical size relationships. L1 is not shown. VPM, ventral posterior medial nucleus of the thalamus; POm, posterior medial nucleus of the thalamus.

connections to and within BC is again simplified and does not take into account interactions between barrels and septa, which receive extralemniscal inputs, as well as other local and long-range inputs.

The predominant cortico-cortical long-range projections from BC come from L2/3, targeting S2 and M1 in particular (Fig. 1.3D). Some authors (Bureau et al., 2006) differentiate within L2/3 a lemniscal, touch-representing circuit projecting to S2 and a paralemniscal, whisking-representing circuit projecting to M1. As in other primary sensory cortices, L5 is the main source of projections to subcortical structures, which include the striatum and brainstem nuclei, in particular the trigeminal nucleus. While it is known that L6 is involved in feedback loops with the thalamus, its connectivity patterns are as yet less clear.

It should be mentioned that BC has become the area of choice for many questions which are only indirectly related to sensorimotor processing. This includes plasticity (Wilbrecht et al., 2010), development (Matsui et al., 2013), glial functioning (Houades et al., 2008), and neuromodulatory mechanisms (Constantinople and Bruno, 2011). There have also been numerous studies of BC development and function in disease models, including Rett syndrome (Moroto et al., 2013), fragile X-syndrome (Harlow et al., 2010), Alzheimer's Disease (Beker et al., 2012) and, of particular relevance to the study of social representations in BC, autism (Smith et al., 2011). These diverse approaches and the substantial body of information on cortical connectivity and function, molecular mechanisms, and development, might provide a basis to use social touch and BC activity as a multi-faceted and at the same time dissectable model for structure-function relationships in models of autism and other disorders.

## 1.4 Motivation

Sensory neuroscience, and in particular electrophysiology and imaging in animal models, has made great progress in understanding the processing and representation of sensory stimuli for different sensory modalities and for different stimulus properties within these modalities. In somatosensory cortex, since the pioneering studies of Woolsey and Van der Loos (1970), the BC as the somatosensory representation of the vibrissae has been investigated with regard to its processing of manifold stimulus parameters, and comparable studies which try to tease apart representations of different visual and auditory parameters have also been performed in the corresponding primary cortices (Hubel and Wiesel, 1968; Sally and Kelly, 1988). In parallel to this reductionist approach, human studies of sensory cortices, in addition to reproducing some of the findings in animals, have taken a decoding approach, which showed that the properties of complex stimuli can be decoded from activity in early visual areas, including primary visual cortex (Kay et al., 2008; Stansbury et al., 2013). These studies, as well as findings of cognitive modulation of primary sensory cortex activity by previous knowledge of the stimulus (Gazzola et al., 2012; Smith and Goodale, 2013; Bannert and Bartels, 2013), indicate that primary cortices might carry out more complex computations than previously thought (Marr, 1982). The responses of sensory areas to socially relevant stimuli have been so far mostly investigated for pictures of faces shown to monkeys (Perrett et al., 1982; Yovel and Freiwald, 2013). There is, however, only one recent report of social stimulus representation in a primary sensory area, where responses of primary visual cortex to faces were observed using voltage-sensitive dye imaging (Ayzenshtat et al., 2012). Critically, the behavioral relevance of rapidly flashed faces without the other sensory inputs associated with a real animal is questionable. Thus, there is an experimental gap between higher-order processing of complex stimuli as presented to human subjects, but necessarily investigated with low temporal and spatial resolution, and animal studies, which provide this degree of resolution, but have so far concentrated on relatively simple stimuli. The present study aims at narrowing this gap by recording from single units in BC, while rats are investigating complex stimuli which are, in addition, intrinsically relevant, i.e., they have a value for the animal independently of experimentally provided rewards. If complex stimuli evoke emerging representations in sensory cortices which are more than the summation of responses to different low-level stimulus properties, the rodent somatosensory cortex as the arguably neurophysiologically best-described sensory system should be the appropriate area to investigate these representations.

Such gains in knowledge could be generalizable on two levels. On the behavioral level, other sensory systems could serve similar purposes and thus be governed by similar principles. For example, as already pointed out by Carvell and Simons (1990), the whisker active touch system is similar in many ways to the use of finger tips of primates. And on the neuronal level, computations in primary sensory areas are thought to be at least partly implemented in canonical circuits found in different sensory cortices (Douglas and Martin, 2004).

As BC is such a well-studied brain area, and rat social behavior has also been investigated in depth, it is additionally conceivable to take the neurophysiology of rat social behavior

as the basis to develop and test models of diseases which affect social behavior. The most prominent in this regard are autism spectrum disorders, for which several rodent models are available (Pletnikov et al., 1999; Schneider and Przewlocki, 2005; Greco et al., 2013). While these disorders are very common and well described on the symptomatic level, no causal intervention is possible. For a mechanistic explanation of the corresponding disease mechanisms, a characterization of molecular mechanisms is key, and great progress has been already achieved. To translate the results into the clinic for such a complex disorder, it might, however, be necessary to describe the network and cognitive effects of putative interventions. The knowledge gains on social representations in the healthy rodent brain might thus in the long run serve to investigate the pathological changes in disease models, and how they can be reversed by possible interventions.

## 1.5 Goals and hypotheses

The goal of the present study was to characterize the representations of complex social stimuli in rat primary somatosensory cortex, considering the subject and stimulus animal sex. The low-level hypothesis was that social touch would be associated with reliable response changes in neuronal activity. This was addressed by comparing firing rates during interactions with baseline firing rates, as well as by recording from whisker-trimmed animals. Starting from this, the questions to be addressed were: How do responses to social touch compare to the touch of other stimuli? Does the sex of the subject and/or the stimulus rat have an influence on neuronal response patterns during interactions? Does the estrus state play a role in how female rats repesent social touch? And if differences are found, are they due to differential whisking behavior and can thus be explained by differing mechanical inputs? Or are there indications of additional influences on neuronal activity? Although this was not the initial focus of the study, it was later attempted to go beyond pooling of all neurons, and towards investigating how the response patterns vary between neurons of different type and layer, as well as neurons with different RFs.

# 2 Methods

## 2.1 Subjects, paradigms, and stimuli

### 2.1.1 Experimental setup and definition of interaction events

During social interactions rats were placed on metal platforms within a Faraday cage (Fig. 2.1). The elevation of the platforms from the table surface was approximately 20 cm, sufficient to avoid the rats from jumping down. In fact, even those rats which fell down once, were apparently too afraid to actively jump down afterwards. The platforms were 30 cm long and 25 cm wide, and were surrounded by walls of 35 cm height. Only rarely did rats jump onto the walls. The typical distance between the subject and stimulus platforms was 20 cm, although it was varied between 16 cm and 22 cm according to animal size. The platforms and walls were covered with soft black foam mats, which provided a dark background for video recordings and helped minimize mechanical and electrical artifacts when the subject rat touched a wall with the headstage. Experiments took place in near-complete darkness, as the room was darkened and the Faraday cage additionally covered with blackout curtains. However, a low level of stray light from computer screens and recording equipment could not be excluded. In early experiments, two LEDs were attached to the headstage for head tracking, in which case the head was brightly illuminated. This was stopped later to avoid illumination and because the tether wire tended to twist around the LEDs. Illumination for observation and video-taping was provided by two infrared projectors (Abus TV6830, wavelength 880 nm), which were positioned ca. 60 cm over the setup.

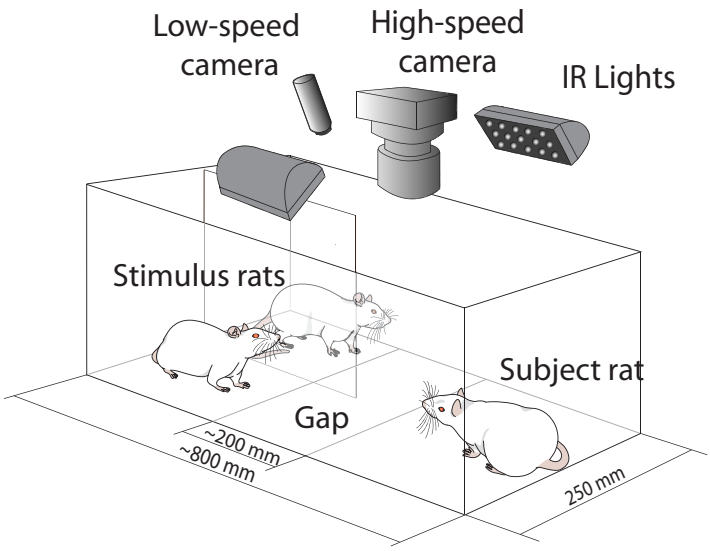



Figure 2.1: Behavioral setup. Depicted is the chooser paradigm, where two stimulus rats were presented in parallel. The scene is illuminated by infrared (IR) lights and recorded continuously with a low-speed camera, as well as with a high-speed camera during interacions.

Behavior was continuously recorded using a camera with either 25 or 30 frames per second with a visible light-blocking filter. These low-speed videos were used to determine the behavioral events relative to which neuronal responses were analyzed: The time of first whisker overlap was defined as the interaction start, and the time of last whisker overlap as the interaction end. Most interactions included very close approaches with the heads of the two rats touching each other, which was also recorded as a behavioral event. The end of head touch was not recorded, but typically occurred within 100 ms before the end of whisker overlap.

To be included in the analysis, an interaction had to last at least 400 ms, and be separated from the next interaction by minimally 400 ms. When the approach was strongly lateralized, which was only rarely the case, the whisker overlap was determined for the subject rat's whiskers contralateral to the implanted hemisphere. In these cases, first whisker overlap and first head touch times could be identical. To ensure sufficient sampling, each stimulus rat had to be interacted with at least three times to enter the analysis based on single stimulus animals. The same restriction applied to the touch of objects, as well as the stuffed and the anesthetized rat.

Stimulus rats were presented either in a chooser setting or alone. In the chooser paradigm (see Fig. 2.1, as well as Wolfe et al., 2011), which was the majority of experiments, two stimulus rats were placed on the platform, separated by a wall. This separation wall was made from thick black styrofoam, covered by black cardboard, and extended beyond the edge of the stimulus platform, so that facial interactions between the stimulus rats were precluded. Nevertheless, the stimulus rats showed behaviors indicative of strong interest in each other, namely biting the wall, sniffing at the wall's edges, and sometimes attempts to climb the walls. Most stimulus pairs were of mixed sex, but in a subset of experiments stimulus rats of the same sex were presented.

Recordings consisted of blocks of 5-10 minutes each. After each block, the light was turned on, the stimulus animals were removed and the mats exchanged to minimize olfactory cues. There were 3-8 blocks in one recording session.

### 2.1.2 Subject and stimulus rats

Subject and stimulus animals were adult Wistar rats aged P50 to P110. Prior to surgery, the subject animals were handled for two to three days to get them used to the experimenter and to being touched and lifted. On two to four further days they were put onto the platforms in the setup for ca. 20 min for habituation. The same procedure applied to the stimulus animals. Extracellular recordings were performed in eight female and six male Wistar rats. Stimulus animals were in the same age range as the subject animal or slightly older. After surgery, subject animals were housed singly, while stimulus animals remained housed in same-sex groups of two to three per cage. No contingent record was kept of whether the subject rat had been in one cage with the stimulus animals previously, or whether they were even littermates. Thus, stimulus animals cannot be categorized by their novelty. However, even if known, this would only apply for a small subset of recordings, as the stimulus animals were

reused over days and were thus nearly always known from day two onwards.

All animals were kept on a 12:12 reversed light/dark cycle with lights off at 8 a.m. and had free access to food and water.

All experimental procedures were performed according to German guidelines on animal welfare under the supervision of local ethics committees according to animal experimentation permit G0259/09.

### 2.1.3 Whisker trimming

In a subset of experiments, the whiskers were trimmed. Trimming took place in two ways. First, in three animals, it was possible to trim the whiskers during a recording session and acquire interaction data before and after trimming from the same units. This was only possible if the animal was very well trained and remained calm during the trimming procedure. In this trimming, macrovibrissae stumps of $<$2 mm and the microvibrissae remained uncut. At a later stage in these three, as well as in two further animals, the macrovibrissae and larger microvibrissae were trimmed under isoflurane anesthesia so that no stumps remained. For trimmed animals, behavioral events analogous to the whisker overlap start and end, as used for untrimmed rats, were defined as the time points when the whiskers would have presumably started or stopped overlapping, if they had been present. Naturally, these "virtual" events can only be approximations. However, in a vast majority of cases there was also a head touch event, so that purely virtual interactions were an exception.

### 2.1.4 Stuffed rat as stimulus

In both purely behavioral and physiological experiments, a stuffed rat was included as a control stimulus. The stuffed rat was a taxidermized adult male rat. The main tactile differences to alive rats were the stiffness of the body and skin, and the angle of the whiskers. While alive rats either whisked actively or held their whiskers only slightly retracted, the stuffed rat had its whiskers in a very retracted position (ca. -45°, with 0° being perpendicular to head midline). The stuffed rat did not have any distinctive smell to the human observer, but clearly lacked the strong smell of adult male rats.

The positioning of the stuffed rat was such that the nose was approximately in the middle of the gap between the platforms, and could always comfortably be reached by the subject rat. As the subject rats occasionally bit the stuffed rat and pulled it down from the platform, in later experiments the stuffed rat was additionally fixed on the stimulus platform.

### 2.1.5 Objects and anesthetized rat as stimuli

In physiological experiments, objects and an anesthetized rat were presented as further control stimuli. The objects presented were most often cuboidal, such as blocks made from polystyrene, cardboard, wood, or a mix of cardboard and paper (i.e., books). In some cases, cylindrical objects as plastic bottles or toilet paper rolls were presented. The objects were positioned on the platform opposite to the subject animal in a fashion similar to the stuffed

rat, such that the object extended approximately to the center of the gap between platforms. Objects changed over the days of one experiment to increase interest in and the number of interactions with them. In some cases, the subject rats tried to climb onto the objects and over onto the opposite platform, so that interactions had to be either interrupted, or the presentation of the object in question had to be stopped altogether.

The anesthetized rat was an adult male rat of ca. 350 g weight. It was anesthetized with ketamine and xylazine, similar to the subject rat at the beginning of surgery (see 2.3.1), and was then presented to the subject rat in a location similar to the stuffed rat and objects, i.e., in the middle of the gap between platforms. However, it was hand-held by the experimenter, and in some cases the snout or anogenital region of the anesthetized rat were brought close to the subject rat to rouse interest. This typically resulted in active whisking onto the anesthetized rat, and a bout of interactions even after the anesthetized rat had been brought back to its normal position over the gap.

### 2.1.6 Comparison of stuffed with alive rat interactions

From here on, the term 'alive rat' denominates an awake, behaving rat, as opposed to other, immobile stimuli, including the anesthetized rat, although this was, of course, also alive.

**Paradigms** A stuffed rat was presented either alongside alive rats in a chooser paradigm, or alone. In behavioral experiments explicitly aimed at comparing interactions with stuffed and alive rats, these stimuli were presented together in a chooser setting. On each day, two recordings of five minutes length were performed for each subject rat, which could be either male or female. Between these two recordings, the alive stimulus rat, which was always male, was changed. The position of the stimuli on the platform was pseudo-randomized.

In physiological experiments, to maximize the number of interactions with the stuffed rat while minimizing the presentation time, the stuffed rat was presented alone in nearly all cases. In addition, presentation blocks could either be shortened when the subject rat showed no interest in the stuffed rat at all, or lengthened to increase the number of interactions. Furthermore, stuffed rat presentations were not distributed evenly within recordings, with an above-average probability of being presented amongst the last blocks on a given recording day. These adjustments to other experimental needs made interaction times with the stuffed rat from experiments with neuronal recordings difficult to compare with alive rat interaction times. This limitation did not apply to the purely behavioral experiments, however.

**Quantification of behavior in chooser settings** The behavior during purely behavioral stuffed vs. alive chooser experiments was quantified by scoring the subject rat behavior in the low-speed videos with a custom-written Matlab script. The scoring was applied in intervals of 0.67 s (corresponding to 20 frames), with five possible behavioral states: (1) subject rat interacting with alive rat, (2) subject rat in the proximity of the alive rat, but not touching it (typically over the gap, but sometimes orienting towards the stimulus while remaining

completely over the platform), (3) no orientation towards either stimulus, (4) subject rat in the proximity of the stuffed rat, and (5) subject rat interacting with the stuffed rat.

## 2.2 Receptive fields and whisker set angles

### 2.2.1 Mapping of receptive fields

Receptive fields (RFs) were determined in most experiments by hand-mapping. For this, whiskers were touched with a thin metal rod, while listening to one of the high-pass filtered continuous acquisition channels for each tetrode. Thus, strictly speaking, the mean RF at a certain recording location, and not the RF of any specific unit was determined. While very different RFs for adjacent cells in barrel cortex are highly improbable (Simons, 1978; Kerr et al., 2007), a certain variation on the scale of under 100 µm might have been missed, particularly at the border of barrels with septa. To avoid additional stress for the animals before recordings, mapping was done after recordings had ended. The quality of mapping strongly depended on the quietness of the animal, so that if the animal was very nervous or stuck its head into the corner, mapping was often approximate, and was sometimes skipped completely. In some cases, RFs could be mapped to single whiskers, but typically this was not possible. The predominant reason was presumably the limited time that the animal would remain quiet, while RFs from several tetrodes had to be mapped. However, RFs in BC can also be diffuse, especially in the septa (Woolsey and Van der Loos, 1970; Brecht and Sakmann, 2002). Typically, the responses seemed equally strong for several whiskers, and in these cases RF positions were calculated as means of the constituent whisker positions. Maximally six whiskers could be given equal weight. Whiskers which showed weaker responses than the principal whisker(s) were not considered in RF calculations. In some cases, RFs could not be determined for a certain recording site, but were determined for adjacent recording sites on the previous or subsequent day on the same tetrode. In these cases, RFs were assumed to remain approximately stable. Predominantly, the RF locations of recorded SUs were in the lower posterior part of the whisker pad (Fig. 3.2). No RFs with centers above the C row were recorded. Only in very few cases did neuronal responses not show any relation to whisker touch. These were included in the analysis, if the recordings from this tetrode on other days showed clear relation to whisker touch. Most responses were clearly indicative of the postero-medial barrel subfield, only in a few cases did units also respond to the snout microvibrissae, nose touch, or touch of the skin posterior to the macrovibrissae. Ipsilateral whiskers were not mapped routinely, but when this was the case, responses were never observed. Section 3.1.1 gives examples of RF distributions for single rats, as well as population data on RF mapping.

### 2.2.2 Correlation of receptive field antero-posterior location and time of peak response

For the analysis of how RF location correlated with the peak time of neuronal responses (3.3.5), all units (i.e., including single and multi-units), were used, but only those which fired at least 1000 spikes during recording time. This threshold was introduced, because for units

which fired at very low rates, spurious peaks in the PSTHs of neuronal responses became increasingly common. The values reported in 3.3.5 are correlations between the locations of RF centers on the antero-posterior axis, and the first bin within the PSTH which reached the maximal response of all PSTH bins. RF centers could be located between whiskers, and these locations were used for correlations. However, for better visualization, units have been assigned to six discrete bins (approximately corresponding to columns) in Fig. 3.37. Receptive fields in column 1 or posterior to it were attributed to column 1, every unit with RF center in column 2 or between 2 and 1 was attributed to 2, and so on for columns 3-5. Units with RF centers anterior to column 5 were pooled and labelled 'ant.' for 'anterior'. The peak bins were determined in PSTHs which included 1000 ms before and after interaction onset or offset, respectively, and were binned with a bin width of 100 ms. In cases where several bins reached the same maximal firing rate, the first of these was taken as the peak.

### 2.2.3 Analysis of whisker set angle over the course of single interactions

For the analysis of whisker set angle (i.e., the average whisker angle) over the course of single interactions (3.3.5), the interactions had to be divided into sampling periods (bins). Bins were distributed over the length of each interaction, such that the first bin began at interaction start and the last ended at interaction end. The average length of interactions where whiskers were tracked was 1.47±0.98 s, and the median was 1.17 s. In accordance with these interaction lengths, bin width (120 ms) and number (5 bins) were chosen such that only for those 10% of the tracked interactions which were under 600 ms long, there was an overlap between the sampled periods. As the minimal tracked interaction length was 440 ms, this overlap was small, never over 20 ms between two bins.

## 2.3 Electrophysiological recordings

### 2.3.1 Surgical procedures

The sequence during surgery was as follows: (1) short anesthesia with isoflurane and injection of 100 mg/kg ketamine and 7.5 mg/kg xylazine as initial dose (100%) (2) shaving the fur on the scalp (3) begin of temperature monitoring using a rectal probe, aiming at 36°C (4) head fixation in the stereotactic setup (5) application of lidocaine on the scalp and cutting of the scalp (6) removal of subcutaneous fat and periostium, as well as attached muscles to get a clean and dry skull surface; cleaning with saline, ethanol, and 1% hydrogen peroxide (7) marking the prospective craniotomy position and the position of reference screws; craniotomy coordinates: 1.5 mm to 3.5 mm posterior to bregma and 4.5 mm to 6.5 mm lateral to midline (8) application of 2-3 drops of glue (Optibond, Kerr, Rastatt/Germany) and hardening it with UV light (9) distributing additional stabilizing glue (Charisma, Heraeus Kulzer, South Bend, IN, USA) on the skull except for the craniotomy and reference screw locations, and hardening it with UV light (10) drilling skull at reference screw sites and position the screws there (11) removing skull above the craniotomy; removing dura; removing blood until the typically observed bleeding stopped (12) positioning the microdrive above the craniotomy

and soldering the ground wire to the screws (13) lowering the microdrive until it was just above the brain surface and sealing the craniotomy with 1% agarose solution (14) fixing the microdrive to the skull with dental cement. During surgery, anesthesia was monitored by respiration rate, whisker trembling, and pinch reflex, and, if anesthesia became superficial, additional doses of 25% of the amount of either the ketamine/xylazine mix or ketamine only used for initial anesthesia were given alternatedly. In 12 out of 14 animals, recordings were performed in the right hemisphere.

### 2.3.2 Microdrive, tetrodes, and spike-channel recordings

The implanted microdrive (Neuralynx 8-drive; Neuralynx, Bozeman, MT, USA) included eight separately movable tetrodes. These were contained within fine polyimide tubes, and bundled by a larger polyimide guiding tube with a diameter of 1.3 mm. The tetrodes were twisted from 12.5 µm diameter nichrome wire coated with polyimide (California Fine Wire Company) and gold-plated to a resistance of 250-300 kΩ in the gold-plating solution. After passing through a unity-gain headstage (Neuralynx), signals were transmitted through a tether cable to a programmable amplifier (Digital Lynx; Neuralynx). The spike signals were amplified by a factor of 10 and then digitized at 32 kHz. The digital signal was bandpass filtered between 600 Hz and 6 kHz. Events that reached a user-set threshold were recorded for 1 ms (250 µs before voltage peak and 750 µs after peak). The threshold was set manually to optimize tradeoff between the desired exclusion of noise and the undesired exclusion of spikes. Thus, if the signal-to-noise ratio was low, a part of the spikes from recorded units could be missed. This was indicated during spike clustering by cluster shapes with sharp linear borders instead of an oval shape. Typically, the threshold for spike detection was set between 50 and 70 µV.

### 2.3.3 Spike sorting and clustering

Spikes were sorted off-line on the basis of their amplitude and spike shape by means of a semiautomatic clustering algorithm (KlustaKwik; 2000; written by K. D. Harris). The resulting classification was corrected and refined manually with MClust software (written by A. D. Redish, University of Minnesota, Minneapolis, MN, USA) running in Matlab (MathWorks, Natick, MA, USA). The spike features used for separation were energy and the first derivative of the energy, with a separate value for each of the four tetrode channels, resulting in eight features overall. Thus, the clustering of recorded spikes took place in an eight-dimensional space, and visualizations can only capture a particularly informative subset of all the features used. An example for different spike shapes recorded on the same tetrode and assigned to different units can be seen in Fig. 2.2. These units differred in spike sizes and shapes over the four recording channels.

As can be seen in Fig. 2.3A, some units could be clearly distinguished using just two spike features. The most similar pair in this example consisted of the units depicted in blue and

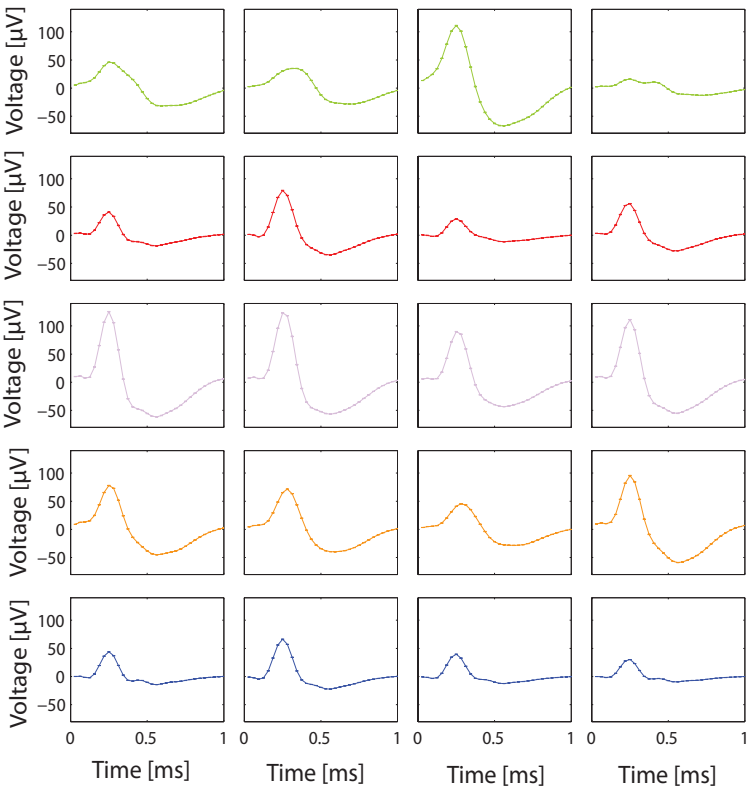



Figure 2.2: Average spike shapes for five single units (rows), recorded on the four channels of one tetrode (columns).

red (also see Fig. 2.2), as indicated by some overlap between the two corresponding spike shape feature clouds ('clusters') in Fig. 2.3. However, Fig. 2.3B also indicates through the independently oval shape of the two clusters and their different orientation that despite some overlap, these two units are distinct, resulting in the classification of these five clusters as single units (SUs), in this case regular-spikers (see 2.3.5).

To be included in the analysis, SUs had to fulfill the following criteria: First, the L-ratio, a measure of distance between clusters (Schmitzer-Torbert et al., 2005), was below 0.5. Second, the histogram of inter-spike intervals (ISIs) was indicative of a SU. This was taken to be the case, if there was either a clear refractory time, or a refractory time was not present, but the histogram sharply decreased after the initial peak, indicating cells bursting with short ISIs. Flat ISI histograms were indicative of multi-unit (MU) activities. Third, a composite criterion for the stability of a recording was applied, based on (a) the firing rate over the course of the recording and (b) the presumable completeness of the cluster (see 2.3.4).

### 2.3.4 Tetrode positioning and recording stability

As described in 2.3.2, the microdrives contained eight tetrodes, which could be separately positioned by turning a screw connected to each tetrode. One turn corresponded to 167 µm,

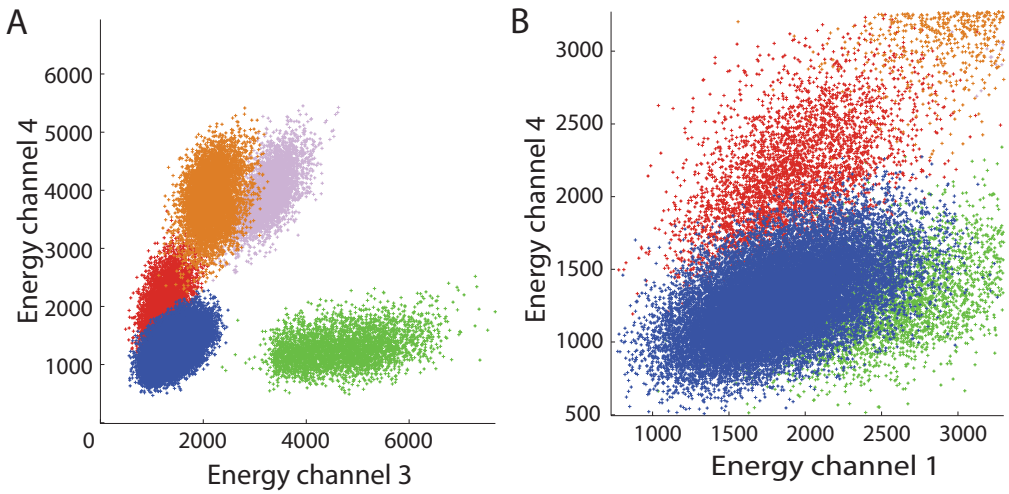



Figure 2.3: A, Comparison of spike energy values from two tetrode channels for the clusters corresponding to the five units in Fig. 2.2. Note that some units can already be clearly distinguished in one plain, but the actual clustering takes place in a high-dimensional space. B, Conventions as in A, but for a different combination of tetrode channels. The red and blue cluster have some overlap, but can be attributed to two different units.

and thus the minimal distance which the tetrodes were typically turned was 42 µm, or a quarter of a turn. Tetrodes were moved before or after the recording, and sometimes both. They were typically turned before recording, if no SUs were present. In some instances, tetrodes were kept in one location over subsequent days, typically when recordings were good and the number of tetrode turns indicated that the location was already in the deep layers. Only in a few instances were tetrodes elevated back towards the cortical surface. The few cases of keeping the tetrode in one place over subsequent days, or moving it back up, could have potentially lead to the same units entering the dataset more than once. To my knowledge, there are no quantitative studies on the percentage of units remaining stable over days in tetrode recordings. What has been shown, however, is that units can stay stable over at least three days in recordings with single wires (Ciocchi et al., 2010), and thus a few instances of double-dipping might have occurred.

The tetrodes were often repositioned before the experiment to improve the signal-to-noise ratio. Instability of the signal must always be considered in neuronal recordings, and the repositioning of tetrodes before experiments was expected to decrease the stability of unit recordings. To control for this source of instability, as well as for the rare cases where units completely disappeared from recordings, possibly being destroyed by the tetrode itself, a measure of firing rate stability was introduced. For its calculation, as many time periods outside of interactions were picked, as there were social interactions that day, and these periods had the same distribution of lengths as the interactions had. Using these periods taken from recordings outside of interactions (i.e., baseline time), the linear correlation between time and firing rate was calculated. This was done on randomly distributed baseline periods, and the average Pearson's $R^2$ value over 1000 permutations was used as a measure of stability.

A higher $R^2$ was associated with a stronger drift of firing rate over the recording. There can be, however, other sources of apparent instability, e.g. firing rate drifts due to changing exploratory behavior or alertness over the course of the experiment. Thus, clusters were additionally rated according to whether their shape was cut, which indicates that some spikes from that unit were missed. Only if more than 30% of the spikes were lost due to poor thresholding according to this estimate, the more conservative stability threshold of 0.15 was used. If the cluster was not strongly cut and it could be assumed that the drift was not due to changing spike shapes, the more liberal $R^2$ threshold of 0.3 was used. Values above that typically indicated that the cell appeared or went completely silent during the recording. For MUs, where cluster completeness could not be meaningfully rated, the less conservative criterion was used.

A quantification of firing rate stability as a function of tetrode displacement is found in 3.1.3. An analysis of the predictive value of the tetrode tip location depth for the assignment of recordings to layers can be found with the histological analyses in 3.1.4.

### 2.3.5 Cell classification

Spike shapes were used to classify SUs into putative fast-spiking (FS) and putative regular-spiking (RS) units. Fast-spiking interneurons are known to have larger and shorter spikes (Barthó et al., 2004). Three measures of the spike shape were used to cluster the units into two groups. These measures were half width, peak-to-trough time, and post-positivity, the latter being the integral of the spike waveform between 0.375 ms and 0.75 ms after the spike peak, normalized by peak voltage. For fast, thin spikes this value could turn positive, while it was negative for slow spikes from putative RS. The separation between the two clusters was drawn manually after preclustering using a k-means clustering algorithm implemented in Matlab. Detailed data on cell classification, as well as an assessment of its validity, can be found in the results section (see 3.1.2). From here on, the terms 'regular-spikers' and 'fast-spikers' are used to describe the two populations obtained by the above assignment. It should be kept in mind, however, that this classification based on purely extracellular measures remains putative.

## 2.4 Histology

After the last recording, rats were deeply anesthetized with ketamine and xylazine, and electrolytic lesions were set along the tracks of the tetrodes with highest SU yield by passing a 10 s, 10 µA, tip-negative DC current through the electrode. Rats were transcardially perfused and brains were fixed in 4% paraformaldehyde, cut in 150 µm-thick coronal sections, and stained for cytochrome oxidase (Wong-Riley, 1979). Typically, lesions were set on three or four tetrode tracks, with a pattern designed to make the lesions as easily attributable to tetrodes as possible (see Fig. 2.4). This included different numbers of lesions per tetrode, as well as different spacing. In addition, the antero-posterior distance of tetrodes, as expected based on the pattern of tetrodes in the drive, was taken into account, and in some cases one of

multiple lesions on a tetrode track was placed below the deepest recording location. From the lesions, the layers of recording were determined using Neurolucida (MBF Bioscience, Williston, VT, USA). The distribution of recorded units over layers can be found in section 3.1.4. There were several reasons, why an assignment of layer was not possible for all units. The most common reason was that a unit was recorded from a tetrode which was not amongst the typically three tetrodes with lesions on their tracks. Further reasons included tetrode wires ripped during the course of experiments, which made lesioning through that tetrode impossible, and ambivalent assignment of lesions to tetrodes.


Figure 2.4: Cytochrome oxidase stain of a coronal BC section. Lesions are marked by black triangles, the tetrode track by a dashed line, and the recording site of the example unit in Fig. 3.16 by a white triangle. Note the intense staining of L4, indicative of primary sensory cortices in general (Wong-Riley, 1979; Anderson et al., 2009), and, in this case, BC.

## 2.5 Estrus measurements

Vaginal smears were collected daily during the experiments, as well as at least two days before starting. Cells were collected by carefully inserting an inoculating loop into the vagina. The collected cells were then stained with haematoxylin-eosin in order to visualize cytoplasm and nuclei. Smears were evaluated under a light microscope, with 10x and 40x objective lenses. Determination of the phase of the estrus cycle was done by proportion of the three types of cells as follows: In proestrus the smear consisted of predominantly nucleated epithelial cells. In estrus the smear primarily contained enucleated cornified cells. Metestrus smears consisted of the same proportion of leukocytes, cornified, and epithelial cells. In diestrus, the smear consisted of predominantly leukocytes (Marcondes et al., 2002). In addition to these four phases, four intermediate phase assignments were used, where the estrus phase could not be clearly determined, and rather corresponded to the transition between two phases. Besides of the assessment of smears, the behavior of the rat was taken as an indicator of the estrus state. In particular, darting, ear-wiggling, and lordosis are typical signs of female proceptivity in estrus (Madlafousek et al., 1976; Erskine, 1989). These were exhibited by females in estrus

both spontaneously and when touched in the lower back region. In the analysis, data were split into the groups 'estrus' and 'non-estrus', where the latter contained all entries except for those where the female had been in estrus according to both smears and behavior.

## 2.6 High-speed videography and tracking

Before experiments, subject and stimulus rats' whiskers were tagged under isoflurane anesthesia (3 to 4%). Tags were constructed by forming a small spherical drop (0.5 – 1.0 mm diameter) of high-viscosity ultraviolet-sensitive epoxy (DYMAX Bluewave 50) on the C2 whisker. After hardening, the bottom half of the epoxy sphere was covered with silver paint and the tag was additionally fixed on the whisker with a small drop of superglue. Illuminated from above, this created a very bright image (Fig. 2.5; also see Wolfe et al., 2011). Typically, tags lasted 3 – 7 days and were replaced, if either the tag came off, or the whisker fell out. The stimulus rat additionally received a black dot on the head to facilitate head tracking. Social interactions were recorded using a high-speed camera (Basler A504k) positioned ca. 65 cm above the gap. During stimulus presentation blocks, the platforms were monitored by the experimenter, and high-speed video acquisition was manually started at the beginning of an interaction. The high-speed video signal was constantly buffered on the PC RAM, which allowed to additionally capture the 2 s before the signal for recording initiation was given. All high-speed video was acquired at 250 Hz with 1280 x 1024 pixels. Video frames were streamed directly to a National Instruments PCI express card (PCIe 1429). Video data was buffered on the computer RAM and streamed in parallel to four hard drives, which were configured in RAID 0 mode. Video acquisition was controlled by custom-written Labview programs.

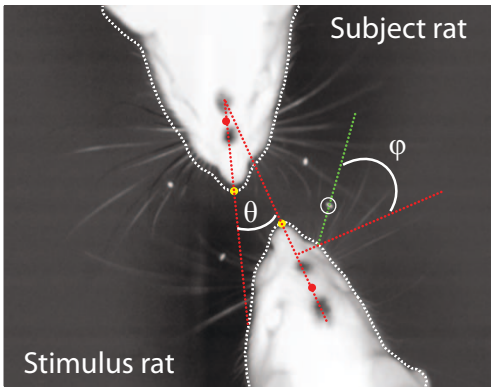



Figure 2.5: Schematic of whisker tracking. After the setting of head center positions (filled red circles), the contours (dashed white lines) were detected automatically. The positions of nose (yellow circles), whisker base (proximal end of dashed green line), and tags (empty white circle) were then set manually. The whisker angle $\varphi$ is defined as the angle between the whisker (a line defined by the whisker tag and whisker base, here the dashed green line) and a line orthogonal to the rat midline. A protracted whisker position corresponded to positive angles. The relative head angle $\theta$ is defined as the angle between the midlines of the two rats.

Whisker tracking was performed using a custom-written code in Matlab, which included the manual setting of the head center position, automated contour detection, and subsequent setting of the nose, whisker base, and tag position. The procedure was similar to Wolfe et al. (2011), but included more manual steps to cope with difficulties caused by the implant and tether cable. Not all interactions could be tracked and whisker tags were not always present. In some cases, whiskers were tracked although tags were missing. The resulting traces were similar to those resulting from tracking of tagged whiskers, but it should be noted that it cannot be excluded that in these cases not always the C2 whisker was tracked, or that different whiskers from the same column were tracked during the course of one interaction. Tracking was done on only one side for the subject and stimulus animals. In most cases, this was the side contralateral to the implant for the subject rat (i.e., the left side) and the opposite side for the stimulus animal, which was mainly the one in contact with the tracked subject whisker.

Whisker tracking was performed for a subset of 200 social interactions, which constitute the dataset in Figs. 3.41-3.43, as well as in Figs. 3.62 and 3.63. The days to be analyzed in depth were chosen to maximize the number of recorded units, while also having a sufficient number of interactions, minimally ten per day. Within days, those interactions were preferably tracked where the whiskers were well visible, i.e., the rat heads were not twisted, and the tag was not occluded for long periods of time. Overall, a balance of units from males and females, as well as of interactions with male and female stimulus animals, was preserved.

## 2.7 Derived measures and further calculations

### 2.7.1 Moving averages of firing rate

The moving average of a unit's firing rate, as shown in Fig. 3.18, was calculated based on a 5 s sliding window, which was displaced in steps of 1 s. The error bars in this figure are standard errors of the mean, and were calculated as follows: (i) segmentation of the 5 s-window into bins of 1 s each, (ii) calculation of the firing rate for each of these bins, and (iii) division of the standard deviation of these firing rates by the square root of the number of bins in the sliding window.

### 2.7.2 Response indices

Firing rate modulation during interactions with a certain stimulus was quantified by comparing the average firing rate during all interactions with that stimulus with a matched baseline period. Stimuli in this sense could be all alive rats, rats of a certain sex, specific stimulus rats, objects, or a stuffed or anesthetized rat. The matched period was as long as the interactions and shifted 10 s relative to these on a spike train from which all interaction periods had been removed. Thus, the shifted baseline period was matched in length to the interactions and could include times directly before or after, but not within this or other interactions. If the point in time calculated after shifting was before the start of the corresponding recording block, the baseline period was reintroduced at the end of the block. For example, a shift

relative to an interaction which began 4 s after the start of the recording block resulted in a baseline period starting 6 s before the end of this block. The response index was defined as follows: Response index = $(R_{in} - R_{out})/(R_{in} + R_{out})$, where $R_{in}$ and $R_{out}$ are the interaction and baseline firing rates, respectively.

The index used in the analysis of correlations between whisking parameters and neuronal responses (section 3.3.13) differred from the above definition. Here, the baseline time was defined as the whole time during which the stimulus in question was presented, except for the interaction phases with any stimulus which occurred during this time. This was necessary for the analysis by single interactions, because otherwise for these short sampling periods there was a high probability of not getting any spikes in the baseline time, and thus producing indices of +1, or not getting any spikes in either interaction or baseline time, so that no index could be calculated at all.

The baseline firing rate values reported throughout this work refer to overall baselines, not the short baseline periods matched to interactions in length.

### 2.7.3 Firing maps

Based on head and nose positions extracted during tracking, the neuronal response could be analyzed in a space centered on the subject rat's head. To this end, the vector from the head position of the subject rat to its nose was rotated such that it came to lie on the ordinate of a coordinate system originating on the subject rat nose. The respective head direction vector of the stimulus rat was rotated correspondingly. This allowed an analysis of neuronal firing relative to the subject head position, as well as a quantification of relative head positions during interactions.

To relate nose position of the two rats to firing rate, color-coded occupancy and firing maps in subject head-centered space were plotted. The steps for the calculation of the occupancy map were as follows: (i) space was discretized into 2D square bins (0.021 x 0.021 cm), (ii) then it was determined which bins the rat had been in how often, and (iii) multiplied by the interframe interval (4 ms) to get the time that the rat spent in that bin. Then (iv) these position data were smoothed with a Gaussian kernel and finally (v) pooled over recording days. For the example unit in Figs. 3.39 and 3.40, bin size was set to 0.043 cm and the smoothing kernel width was 0.085 cm; for the population data in Figs. 3.41 and 3.42, bin size was 0.021 cm and kernel width 0.043 cm. The firing rate map was calculated analogously, in this case counting the spikes in each bin, and then divided by the occupancy map and the number of clusters. To remove excessive smoothing artifacts at the edges, occupancies were not plotted for the positions in the firing rate map which corresponded to the lowest 10% of firing rate values.

## 2.8 Statistics

**General remarks and conventions** Most of the data were not distributed normally, as indicated by the Lilliefors test. Thus, non-parametric statistics are reported throughout this work. In figures, asterisks mark significance levels, with one, two, and three asterisks representing alpha levels of below 0.05, 0.01, and 0.001 respectively.

The values indicating the dispersion of distributions around their means are standard deviations.

**List of applied tests** The following tests were used:

- Wilcoxon signed-rank test: test for the probability that two *paired* samples come from distributions with equal medians, or the probability that one sample's median is not different from zero; referred to as 'signed-rank test'
- Mann-Whitney U test: test for the probability that two *unpaired* samples come from distributions with equal medians; referred to as 'U test'
- Brown-Forsythe test: test for the equality of the variances of two distributions (homoscedasticity); referred to as 'Brown-Forsythe test'
- Kruskal-Wallis one-way analysis of variance: test for the probability that from a group of samples all come from the same distribution; referred to as 'Kruskal-Wallis test'
- Hartigan's dip test of unimodality: test for the probability that a distribution has only one mode; referred to as 'Hartigan's dip test'
- Significance levels for the Pearson's product moment correlation were computed by a transformation of the correlation into a t-statistic, as implemented in Matlab; referred to as 'Pearson's correlation'
- Significance levels of responses at unit level were calculated by a permutation test, which was performed as follows: (i) periods with the same lengths as the available interactions were randomly chosen within the time outside of interactions, (ii) the firing rate during this matched baseline period was calculated, (iii) this permutation was repeated 1000 times, (iv) the distribution of these baseline firing rates was normalized by subtracting its mean, and made one-sided by taking the modulus of these differences, (v) the in-interaction firing rate was similarly normalized by subtracting the mean of the baseline firing rate distribution and taking the modulus of the resulting difference, and (vi) the rank of the normalized in-interaction firing rate within the normalized distribution of baseline firing rates was determined.

# 3 Results

## 3.1 Methodological aspects

### 3.1.1 Whisker receptive fields

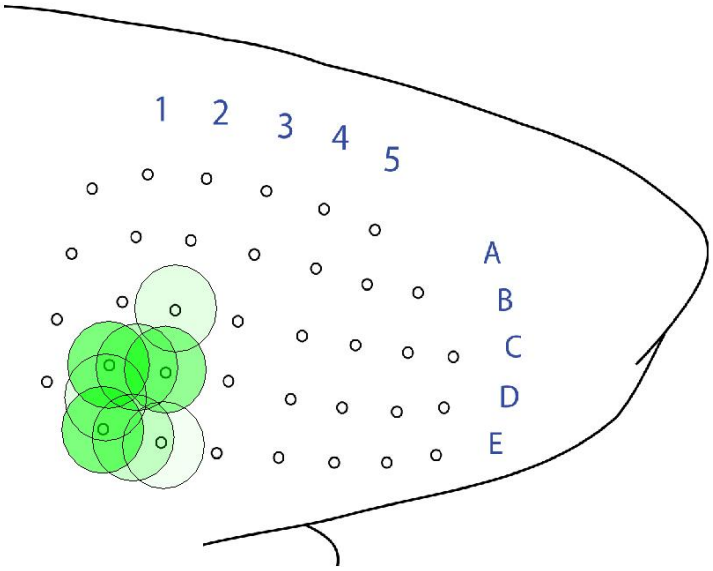
Receptive fields (RFs) were mapped according to the procedure described in 2.2.1. Overall, of 314 SUs recorded when the whiskers of the subject rat were not trimmed, RFs could be determined for 211 (67.2%). Amongst the 32.8% with undetermined RFs, 8.6% were recorded in experiments performed by Rajnish Rao, who did not map RFs, 3.2% were not clearly responsive to passive whisker touch, and 1.3% were not mapped because the animal was unquiet (the percentage of RFs mapped approximately, although the animal was unquiet, was much higher, namely 18.2%). The remaining 19.7% of SUs were responsive to whisker touch, but had no clear RF. Figure 3.1 shows the distribution of RF centers for two example rats, from which many SUs were recorded. Receptive fields were typically similar over both tetrodes and days (Fig. 3.1A), with the exception of one animal (Fig. 3.1B), which showed unusually diverse RFs between both different tetrodes and days, presumably due to oblique tetrode tracks.

Figure 3.2 shows the distribution of all SU RFs. Although RFs sometimes included the straddlers, the most posterior four macrovibrissae, which are not associated with any whisker row, these were never the RF centers. Precise whisker mapping was not possible anterior to the fifth column, so that all units with RFs in very anterior locations have been pooled.

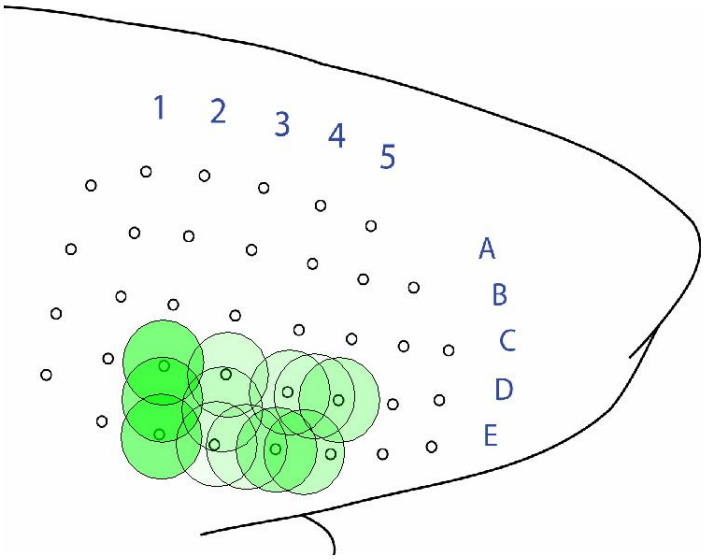
### 3.1.2 Cell classification

As mentioned in 2.3.5, fast-spiking (FS) neurons have been shown to have shorter and larger spikes than regular-spiking (RS) neurons. This could be used by some authors for a reliable classification of SUs from cortical extracellular recordings. When the spike shapes of the neuronal population were analyzed, it was found that the distributions of spike half widths and peak-to-trough times were both significantly bimodal ($P<0.0001$, Hartigan's dip test)[2]. The distribution of post-positivities also appeared to be strongly bimodal (Fig. 3.3A), but this difference was not significant. In addition to the bimodality of the spike shape parameter distributions, the strong correlation between these three measures suggests that they were indicating the same cell type distinction, and were not independently bimodal. Half width and peak-to-trough time were, as to be expected, strongly correlated ($R = 0.668$, $P<0.0001$,

[2]Hartigan's dip test provides a probability that a distribution is not unimodal, and thus does, strictly speaking, not test for bimodality. Therefore, more modes cannot be excluded. However, the a priori assumption of bimodality and the appearance of the distributions strongly suggest that the distributions were bimodal. What is more, even if they were multimodal, this would not argue against the presented cell-type distinction, which is based on the two most pronounced modes.




(a)




(b)

Figure 3.1: RFs of all SUs recorded in two example rats. (a) RFs were typically similar across days and tetrodes, indicating that all recordings in one animal took place from adjacent barrels, as was the case for this animal. (b) In one unusual animal, a wide distribution of RFs over tetrodes and days was observed. The RFs are indicated by circles positioned at the RF center, but circle radius does not correspond to RF size. The intensity of the green color indicates the number of units recorded in the respective location.

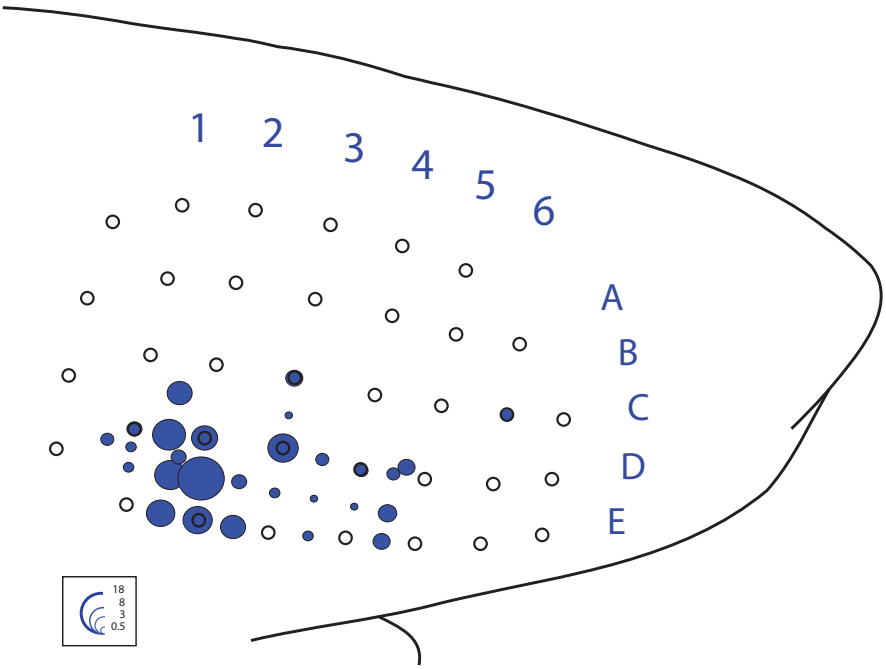



Figure 3.2: Distribution of RF centers of recorded SUs over the whisker pad. Units depicted to have a RF on the C6 whisker include all units with RFs anterior to the fifth column. Scale bar numbers indicate percent of all units with determined RFs.

Pearson's correlation), and, importantly, there was also a strong correlation between post-positivity and both peak-to-trough time ($R = 0.804$, $P<0.0001$) and half width ($R = 0.631$, $P <0.0001$). As a consequence, when plotted in three dimensions, two clusters of units were visible. The distinction between these two clusters was corroborated when the same measures were applied as used for spike clustering on the level of individual tetrode recordings. Thus, the L-ratio of 0.021 and the isolation distance of 20.0 would have indicated a clearly separated SU in the corresponding analysis.

Figure 3.3A shows a two-dimensional clustering by post-positivity and peak-to-trough time. The average spike shapes of RS and FS, as based on this classification, show the typical features of the two cell types, and particularly in the late phase of the spike traces, as quantified by the post-positivity, there was little overlap (Fig. 3.3B). A group of 21 SUs had post-positivities indicative of RS and peak-to-trough-times indicative of FS. As the half width was not conclusive, these units were classified as RS, being the bigger group. In fact, they might represent a subgroup within FS or RS, but it is unclear, which neuronal subtype this might be.

The fact that FS due to their short spikes can fire at higher rates than RS does not necessarily mean that they fire at high rates constantly. Nevertheless, higher average firing rates in FS are to be expected, and were found after dividing the units in these two groups based on the independent measures of spike shape (for FS: mean firing rate = $6.89 \pm 9.75$ Hz,

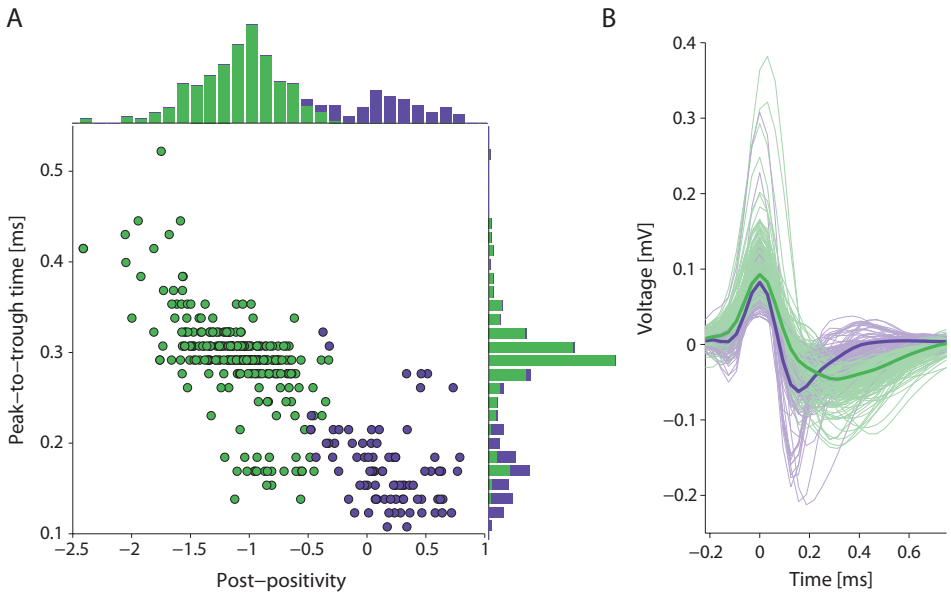



Figure 3.3: Separation of SUs into putative RS (green) and putative FS (violet). A, The distributions of spike shape characteristics were bimodal. This was particularly clear for the peak-to-trough time and post-positivity (for calculation see 2.3.5). For cell identity assignment, the half width of spikes was additionally considered. B, RS had broad spikes with long after-hyperpolarizations, FS had narrow spikes and short after-hyperpolarizations.

n = 72; for RS: mean = 2.78±3.76 Hz, n = 242; P = 0.007, U test; see Fig. 3.4). At the same time, some putative FS fired at very low rates, as is reflected by the fact that the median firing rates were much lower than the means, which was also the case for RS (median = 2.64 Hz for FS and 1.21 Hz for RS). Firing rate was also weakly but highly significantly correlated with each of the spike shape parameters (R between 0.30 and 0.37, $P < 0.0001$ for all three parameters).

### 3.1.3 Tetrode positioning and recording stability

Repositioning of the tetrode immediately before recording start was associated with a decrease in stability, as measured by a drift in baseline firing rate (for a description of its calculation, see section 2.3.4). By this measure, a higher value indicates a higher drift in firing rate, and thus a decrease in stability. The median stability for all SU and MU was 0.100 in cases without displacement (n = 221) and 0.148 with displacement (n = 425), and this difference was highly significant ($P<0.0001$, U test). At the same time, the correlation between tetrode displacement and instability was very small (R = 0.070) and insignificant (P = 0.074, Pearson's correlation). This was partly explained by the surprising finding that amongst those units where the tetrode had been displaced before the recording, a larger displacement was negatively correlated with instability, as can be seen in Fig. 3.5. In other words, if the tetrodes were displaced by a larger amount, this had a beneficial effect on recording stability, as compared to small displacements. This correlation (R = -0.092) was stronger than the

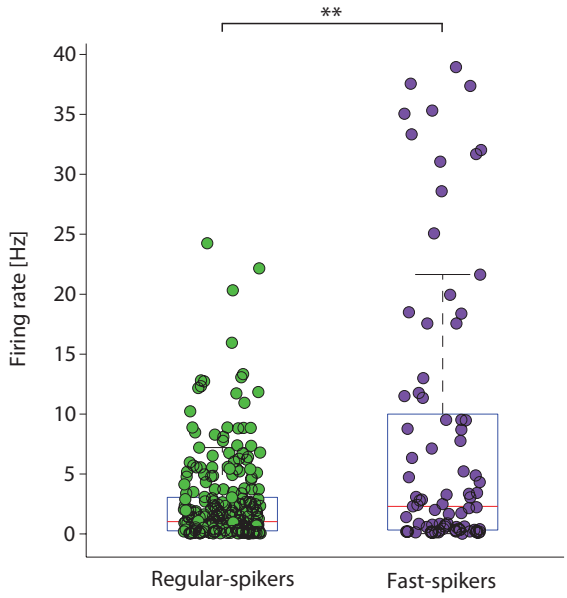



Figure 3.4: Units classified as FS had on average higher firing rates than putative RS. All units firing above 25 Hz were FS. Note that the FS population also included cells with very low firing rates.

positive correlation including all units recorded independently of displacement and was close to significance (P = 0.058, Pearson's correlation). It can be speculated that this is explained by properties of tissue adhesion to the tetrode, such that the tissue remained attached to the tetrode for small displacements, and then relaxed slowly, while the tetrode was immediately repositioned relative to the tissue for larger displacements.

### 3.1.4 Assignment of units to layers

In 60% of all RS neurons, the layer of the recording site could be determined. There was a bias towards layer 5B (L5B), while particularly L6 was underrepresented. Also, no L1 units were recorded at all. These patterns were probably due to both the time course of tetrode placement and the high firing rates of layer 5B cells (see 4.3.2). The distribution across layers for RS, as shown in Fig. 3.6A, was as follows: L1 - no units, L2/3 - 23, L4 - 25, L5A - 32, L5B - 65, L6 - 6. The recording sites as verified by histology were consistent with the RFs described above, insofar as most of the sites were localized in the postero-medial barrel subfield, which corresponds to the macrovibrissae. As Fig. 3.6A also shows, the number of FS recorded was substantially lower than the number of RS, and the number of MUs was between the two. However, it should be kept in mind that these numbers refer only to those units, to which a layer could be assigned based on histology. The overall dataset consisted of a much higher number of units (e.g. 242 RS overall vs. 151 RS with layer assignment), and had a slightly lower percentage of FS (23% vs. 27%).

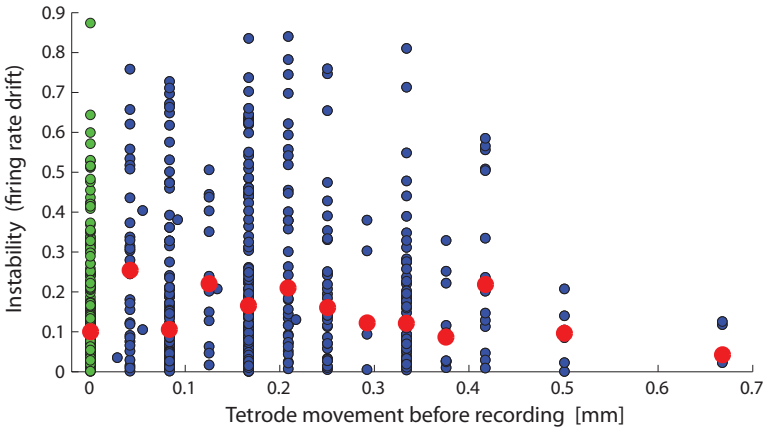



Figure 3.5: Recording instability as a function of tetrode displacement before the start of recording. Positive tetrode movement indicates lowering the tetrode. Units marked in green were recorded without any tetrode displacement before the recording. Red circles indicate medians. Three stability values from instances where the tetrode was elevated before recording have been omitted from the figure, but entered statistics, for which the absolute values of displacements were used.

The contribution of different layers to the dataset with verified histology did not differ strongly between the three types of units recorded (Fig. 3.6B), with the exception of L6, which contributed only 4% of RS, but 16% of FS. There were, however, considerable differences between the contributions of different layers to the datasets recorded from male and female rats (Fig. 3.6C). The data shown here and in Fig. 3.6D refer to RS only, as only RS have been used for the analysis of sex-specificity of BC activity in section 3.3.12. Thus, within the RS with verified layer, the male dataset (n = 90) was heavily biased towards L5B (62%), while L5B contributed only 15% in recordings from females (n = 61). Layer 5A was also slightly more represented in males, while in females the contributions from L2/3 and L4 RS were dominating. In contrast, when the RS were parsed by the estrus state of the subject female, contributions of different layers were roughly comparable (Fig. 3.6D), although L2/3 had a higher contribution in estrus (45%) than in non-estrus females (24%). However, the numbers on layer contribution as a function of estrus state should be taken very cautiously, as from the 32 RS recorded from females in estrus, only 11 had a verified recording layer, such that even the L2/3 contribution consisted of only five units. The corresponding dataset recorded in non-estrus females included 37 RS.

As histology was not available for all animals and all tetrodes, it is interesting to ask whether the laminar location of a neuron could be reliably determined from the recording depth, as deduced from the number of tetrode turns. For this analysis, recording depth was calculated relative to the first location where spikes were observed. As Fig. 3.7 shows, there is the expected very prominent correlation of recording depth with cortical layer ($R = 0.568$, $P < 0.0001$, Pearson's correlation). However, it is also obvious that such an assignment would

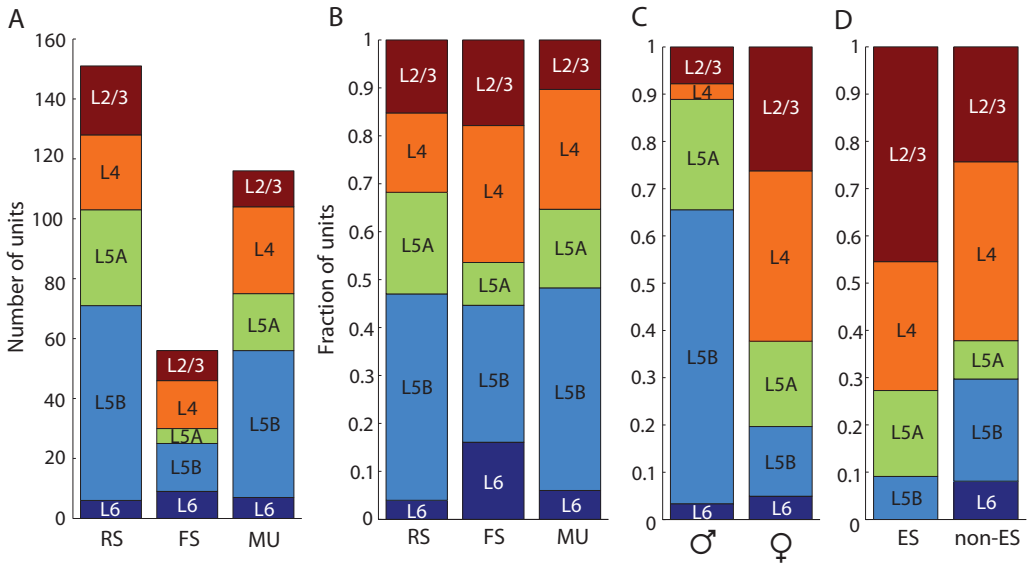



Figure 3.6: Contributions of the units recorded in different layers to the overall dataset with layer assignment. A, Absolute numbers of units from different layers for RS, FS, and MUs. No L1 units were recorded. B, Normalized display of the data in A, showing the relative contributions of the different layers for each type of unit. C, Relative contributions of different layers to the RS recorded in males and females, respectively. D, Relative contributions of different layers to the RS recorded from females in estrus and non-estrus, respectively.

be very imprecise. For a discussion of how this could be improved and made useful, see 4.1.5.

Histology also showed that a very small group of units (n = 4) was inadvertently recorded in the striatum. An example of a striatal interaction-related response and a short discussion of this observation is given in an appendix (section 6).

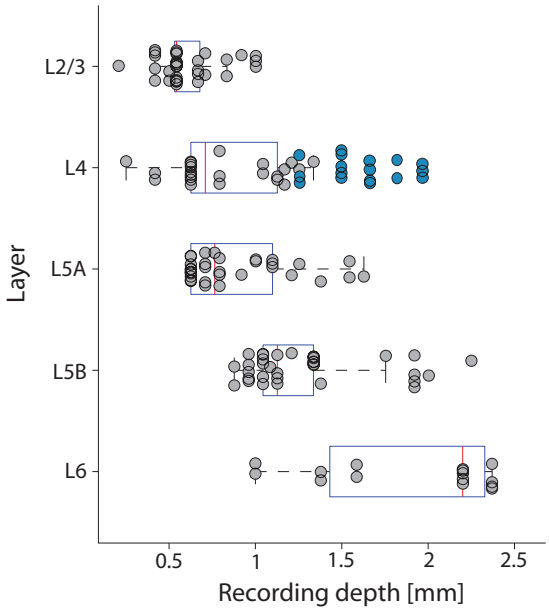



Figure 3.7: Correlation of layers as determined by histology with the depth of the recording location, based on tetrode positioning. A depth of 0 mm corresponded to the first location where spiking activity was observed. Within the units from L4, those recorded in an unusually lateroposterior location constituted a large fraction (17/41). As these were also recorded at larger depths, they are separately plotted in light blue, and were not included for the calculation of the median and quartiles of the corresponding box.

## 3.2 Behavior

### 3.2.1 Patterns of social interactions as a function of stimulus sex

**Female rats as subjects** Female rats strongly and reliably preferred to interact with males. As a measure of interaction preference, normalized interaction times were computed for all interactions with males and females, respectively, as a fraction of the overall presentation time of animals of the respective sex. Thus, a normalized interaction time of 0.05 means that the corresponding interactions covered 5% of the overall presentation time. Normalized interaction times of females were on average 0.047±0.027 for stimulus males, but only 0.021±0.013 for stimulus females (n = 47 days, $P < 0.0001$, signed-rank test). For a recording block length of five minutes, this corresponds to 14.1 s of interactions with stimulus males, but only 6.3 s with females. Thus, females spent more than twice as much time interacting with males as with females (Fig. 3.8A). This preference was found for both estrus and non-estrus females (for estrus: P = 0.007, n = 11; for non-estrus: P = 0.001, n = 19). There was no increase in male preference during estrus. In fact the mean male/female preference ratio (the ratio of normalized interaction times with males and females) was higher during non-estrus (2.62±1.59) than during estrus (2.13±1.26), but this difference was not significant (P=0.464, U test).

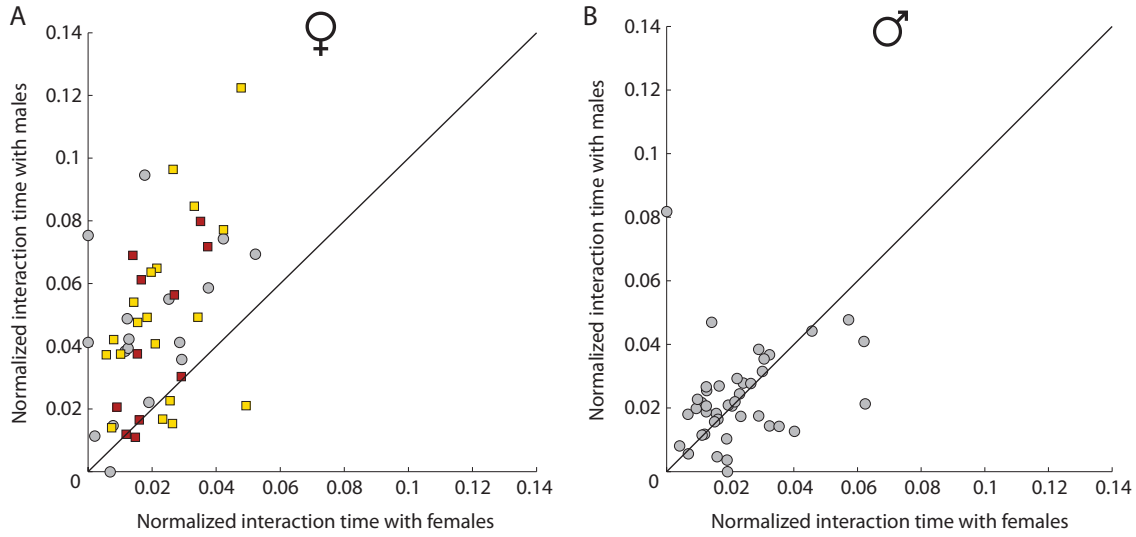



Figure 3.8: The fraction of recordings spent interacting (normalized interaction time) differred by stimulus rat sex for female but not male subject rats. A, For females, normalized interaction times were much higher with males than other females. This effect appeared in both estrus (dark red squares) and non-estrus (yellow squares), as well as for days where subject estrus was not determined (grey circles). B, Same as A for male subject rats. There was no difference in normalized interaction times with male and female stimulus rats. Note that the overall time spent interacting with both sexes was higher for females (A) than for males (B).

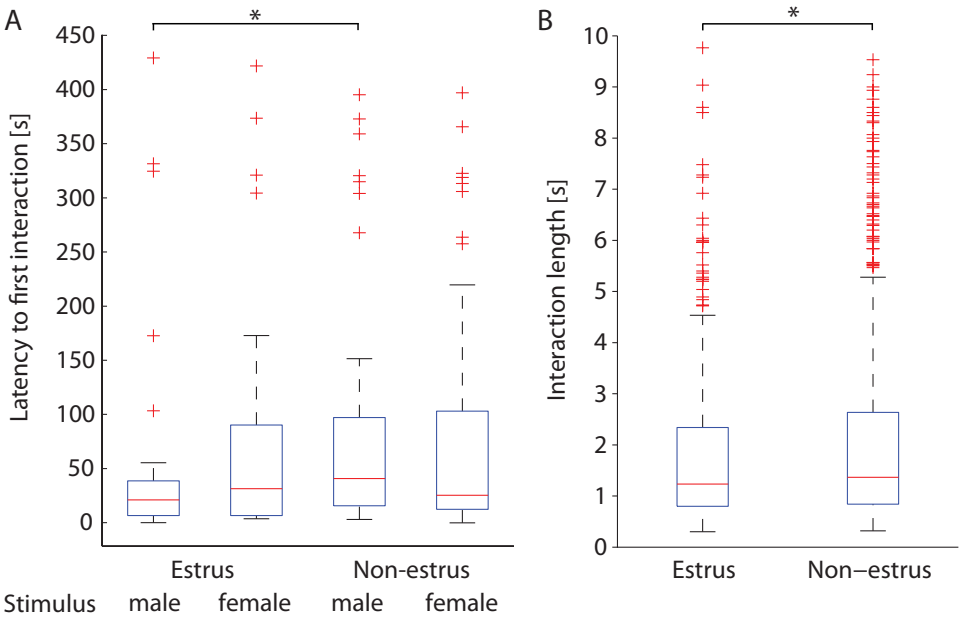



Figure 3.9: A, Latencies of female subject rats to the first interaction with a male were shorter in estrus than in non-estrus. B, Social interactions of females were slightly but significantly shorter in estrus than in non-estrus. The ordinate has been cropped at 10 s.

The latencies of female approach were also lower for stimulus males (mean = 95.2±131.1 s) than females (122.9±146.1 s). This difference was not significant, however (P = 0.436, n = 128 for males, n = 108 for females, U test). There was no effect of subject estrus on sex preference in latencies, as the latencies to male approach were shorter, but not significantly so, in both estrus (P = 0.245, n = 33 for males and 30 for females, U test) and non-estrus (P = 0.358, n = 56 for males, n = 45 for females, U test). There was, however, a sex-specific effect of estrus on the approach latency, such that the estrus state did not influence the latency to female approach (P = 0.799), but lowered the latency to male approach. This latency decreased from 83.8±103.7 s during non-estrus to 56.9±104.5 s during estrus (P = 0.016, n = 56 for non-estrus and 33 for estrus; see Fig. 3.9A). It should be mentioned that, as in other latency analyses pertaining to the chooser paradigm, interest in one animal might have produced higher latencies to interact with the other stimulus partner, such that female stimulus animals might have been approached faster, had they been presented alone.

Interaction lengths of subject females were remarkably similar between stimulus males and females (1316 interactions with males, 574 with females, means 2.06±2.10 s for males and 2.07±2.03 s for females; P = 0.575, U test). Comparing male and female interaction lengths for only estrus and only non-estrus also gave no differences (P>0.3 for both, U test). Thus, there was no effect of stimulus sex on interaction lengths. There was, however, an effect of estrus on interaction lengths (Fig. 3.9B). Interactions became slightly but significantly shorter

during estrus (mean = 2.14±2.21 s in non-estrus vs 1.86±1.82 s in estrus; P = 0.024, n = 844 for non-estrus, n = 457 for estrus, U test). This was in line with anecdotal observations that estrus females often showed bouts of short interactions with rapid approaches and similarly rapid withdrawals, reminding of darting behavior.

**Male rats as subjects** In stark contrast to females, males did not show a preference to interact with the opposite sex (Fig. 3.8B). Normalized interaction times were nearly identical for stimulus females (mean = 0.022±0.015) and stimulus males (0.023±0.015).

Similarly, mean latencies of male approach of male and female stimuli were very similar (66.2±98.8 s for males, n = 108; 59.8±88.8 s for females, n = 103, P = 0.835, U test)

**Comparison of male and female interaction behavior** Females had overall much longer latencies to approach, independent of stimulus sex. The mean approach latency was 63.1±93.9 s for subject males, but 107.9±138.6 s for subject females. Due to the high variation in the data, this difference was not significant, however (P = 0.173, n = 236 for females, n = 211 for males, U test).

Although they took more time to begin interacting, females spent much more time interacting overall. The mean normalized interaction time was 0.052±0.026 for females vs 0.035±0.022 for males (P = 0.0008, n = 42 for males, n = 47 for females, U test; see Fig. 3.8).

### 3.2.2 Other interaction patterns

Over the course of interactions with a certain stimulus animal on a certain day, the length of interactions strongly decreased from the first through the second to the third interaction, before this decrease levelled off (Fig. 3.10). The first interaction had an average length of 3.49±2.48 s (n = 342), while the second was 2.33±2.31 s (n = 323) on average, a highly significant decrease (P<0.0001, U test). There was a further significant decrease of interaction length from the second to the third interaction (mean = 1.89±1.77 s, n = 291; P = 0.011, U test). It should be noted that for this analysis all interactions with a certain stimulus animal on a certain day have been pooled, and there was no difference made between interactions which occurred within one presentation block and those which pertained to different blocks and could thus be separated by long breaks.

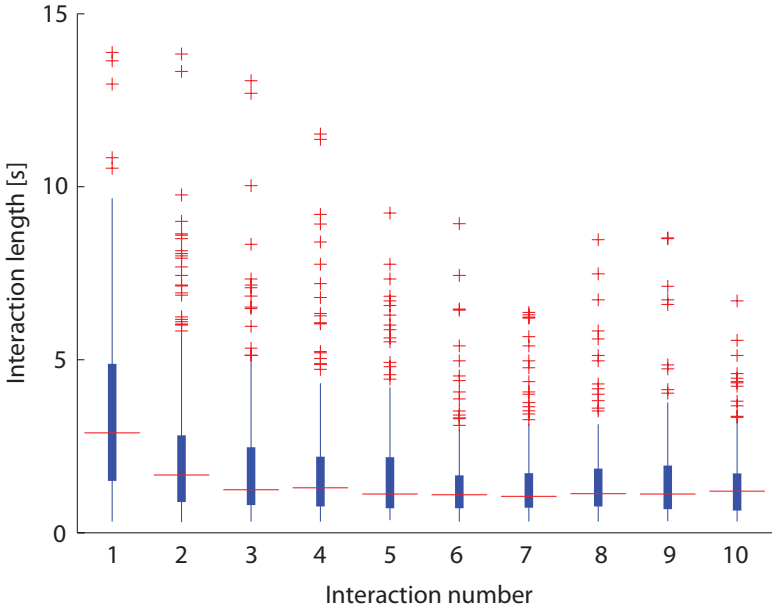



Figure 3.10: Interaction length over subsequent interactions with a certain stimulus animal. The first interaction is the longest, and with the third, interaction lengths level off. In the boxes corresponding to the second and the tenth interaction, one outlier each has been been excluded.

### 3.2.3 Comparison of interactions with stuffed and alive rats: behavioral experiments

To characterize the neuronal responses during social touch, control stimuli were needed to tease apart social from non-social aspects of touch responses. One of these stimuli was a stuffed rat (for details see 2.1.4). As a first step, the behavioral patterns of interaction with stuffed and alive rats were compared. Stuffed and alive rats were presented in a chooser paradigm, and overall 28 periods of five minutes each were recorded on seven days.

On all measures of preference, alive rats were preferred over the stuffed rat. Interactions with alive rats were slightly but significantly longer (alive: mean = 2.59±2.40 s, stuffed: 2.34±2.52 s, n = 144 for alive, n = 116 for stuffed, P = 0.029, U test; see Fig 3.11A). To quantify the time spent in interactions with the stimuli, a normalized interaction time was calculated as the ratio of time spent with a stimulus, divided by the overall recording time. A similar measure was calculated for the time spent in proximity to the respective stimulus. There was a significant preference to engage in interactions with alive rats, with the mean normalized interaction times being 0.045±0.024 for alive rats and 0.032±0.040 for the stuffed rat (P = 0.021, signed-rank test; see Fig 3.11B). The preference to spend time in the proximity of the alive rat was even stronger, with the mean normalized times spent in stimulus proximity being 0.182±0.048 for alive rats and 0.114±0.044 for the stuffed rat (P<0.001, signed-rank test; see Fig 3.11C).

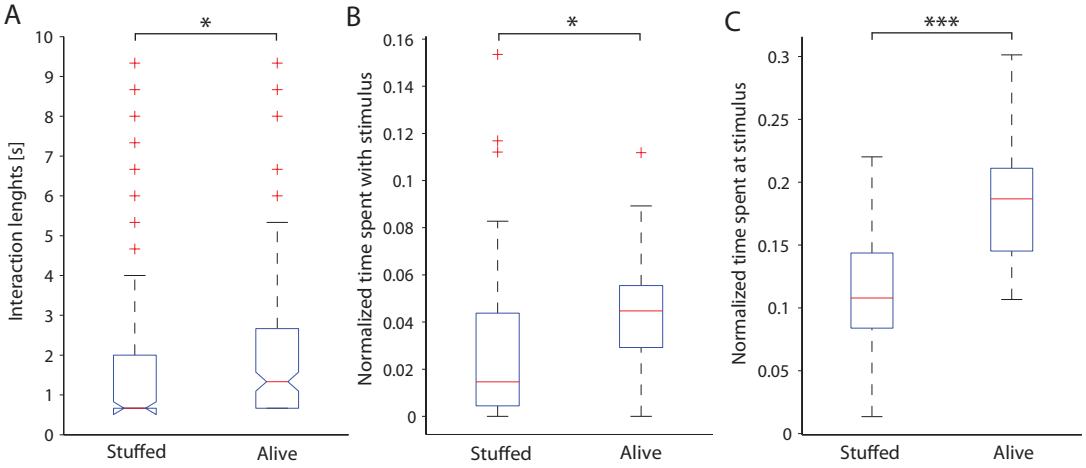



Figure 3.11: Comparisons of interaction and approach behavior for alive rats and the stuffed rat as stimuli. A, Interactions were longer with alive than with stuffed rats. B, There was a preference for alive over stuffed rats with regard to the time spent in interactions ('with stimulus'). C, The preference to spend time in the proximity of alive rats ('at stimulus') was even more pronounced.

Another preference measure is the latency to the first interaction, and here again alive rats were preferred over the stuffed rat. When the complete lack of interactions was included as the maximal possible latency of 300 s, the mean latency was 58.9±82.7 s for alive and 98.4±86.2 s for the stuffed rat ($P = 0.003$, signed-rank test; see Fig 3.12A). The outcome was very similar when recordings with no interactions at all with the respective stimulus were not considered (40.5±49.9 s for alive, 74.3±52.6 s for stuffed, $P = 0.001$, U test).

Finally, there was a trend towards a decrease in the time spent in the proximity of the stuffed rat from the first to the second presentation block for a given day and subject animal. At the same time, such a trend was not observed for alive rats, which were exchanged from the first to the second block (Fig 3.12B). An index which quantified the preference to spend time in the proximity of a stimulus in the first over the second block (1 for being close to the stimulus only in the first block, -1 for only being close in the second), was strongly positive for the stuffed rat as interaction partner (mean index = 0.300±0.648), but negative for alive rats (-0.150±0.446; $P = 0.079$, signed-rank test). This indicates that interest in alive rats was maintained, while interest in the stuffed rat decreased. There is, however, an interrelation between the times available to interact with the different stimuli, so that the negative index for alive rats might be partly caused by high interest in the stuffed rat during the first, as compared to the second block.

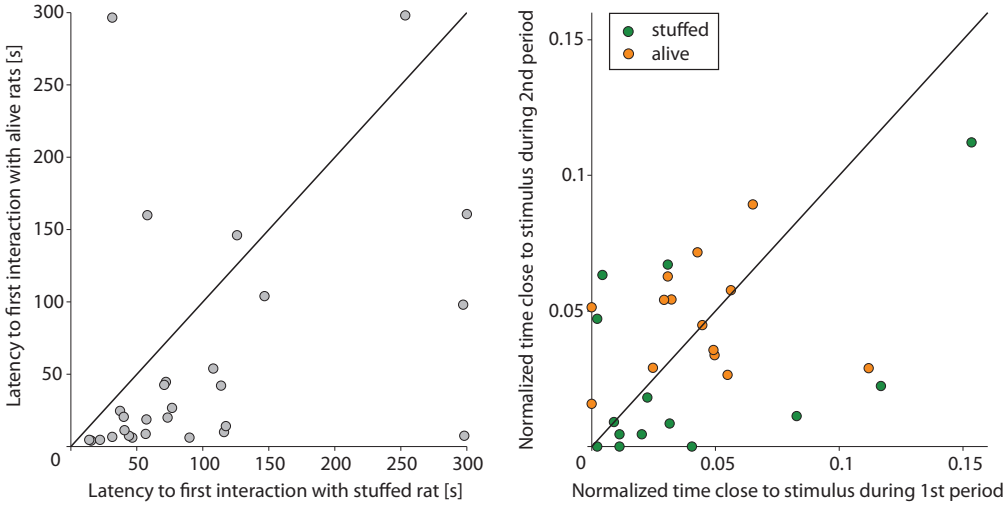



Figure 3.12: Comparison of approach behavior and measures of interest for alive rats and the stuffed rat as stimuli. A, Latencies to the first interaction. Latencies were much shorter for alive rats as stimuli. B, Normalized times spent in proximity to the different stimuli in the first and second presentation period.

### 3.2.4 Comparison of interactions with alive and inanimate stimuli: physiological experiments

The comparison of interactions with alive rats and inanimate stimuli during physiological experiments was complicated by the fact that both stuffed rats and objects were presented more often in the end of the experimental session, which could skew interaction parameters due to fatigue. At the same time, these periods of inanimate stimulus presentation were often shortened if the stimulus elicited no interest. In addition, alive rats were presented in chooser settings most of the time, while inanimate stimuli were presented alone during experiments with neuronal recordings. These factors could potentially bias the normalized interaction times in the other direction. Furthermore, the stimuli differed in their degree of novelty. While over days different objects were presented to a subject animal, and thus objects were typically novel, stimulus animals were used over days, and the stuffed rat was also always the same. This might explain why latencies to first touch were lower for objects (mean latency = 52.3±84.2 s, n = 37) than for alive (86.7±121.5 s, n = 447) or stuffed rat stimuli (74.5±92.3 s, n = 37) (see Fig. 3.13A). The distributions were, however, not significantly different when testing all samples (P= 0.216, Kruskal-Wallis test), and comparing samples individually showed that only the latencies to interact with objects and the stuffed rat were significantly different (P = 0.035, U test). When considering the relatively high latency to interact with alive rats, it should also be noted that both stimulus animals in a chooser setting entered the dataset, such that high interest in one animal might have produced a very long latency to interact with the other stimulus partner.

Despite the above limitations, the behavioral preference to interact with alive over stuffed rats, described in 3.2.3, was reproduced. The distributions of normalized interaction times for the three stimuli were significantly different (P = 0.038, Kruskal-Wallis test), and this was

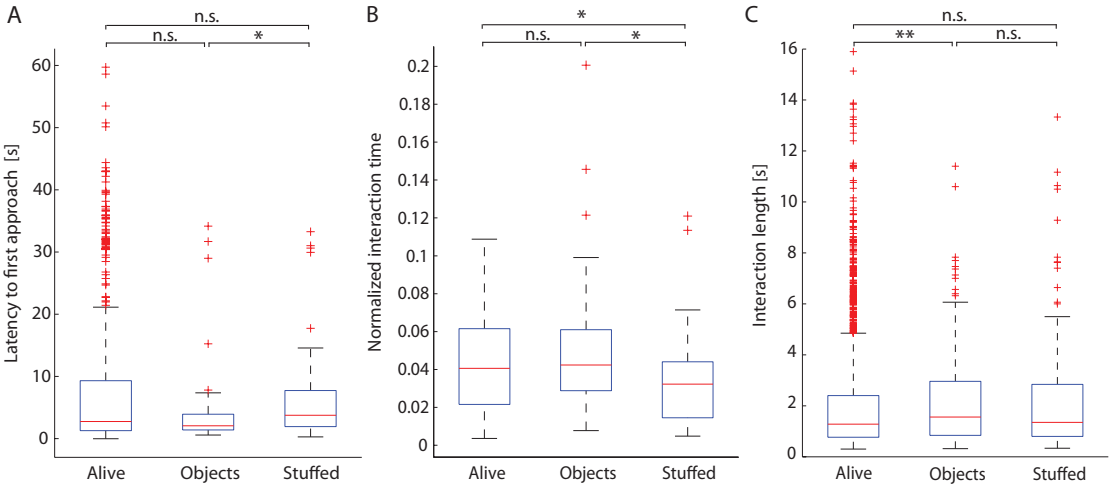



Figure 3.13: Differences in interaction parameters between alive rats and inanimate stimuli. A, Latency to first approach was higher for the stuffed rat than for objects, but did not differ significantly between objects and alive rats. B, The normalized time spent interacting with different stimuli was not different between objects and alive rats, but longer for both than for the stuffed rat stimulus. C, Object interactions were longer than interactions with alive rats. In C, four outliers have been omitted, two for alive rat interactions, and one for stuffed rat and object interactions each. 'n.s.', not significant.

predominantly due to shorter normalized interaction times with the stuffed rat compared to the other stimuli. The normalized time spent in interactions was higher for alive (0.044±0.025) than stuffed stimuli (0.035±0.028; P = 0.035, n = 89 for alive, n = 35 for stuffed, U test). Similarly, objects (0.053±0.040) were touched for longer periods than was the stuffed rat (P = 0.018, n = 36 for objects, U test; see Fig. 3.13B). The preference for alive over stuffed rat interactions was even clearer in a paired comparison for days with stuffed rat presentation (0.049±0.023 vs. 0.035±0.023, P = 0.012, n = 35 days, signed-rank test).

Mean interaction lenghts also differed between the stimulus groups (P = 0.009, Kruskal-Wallis test), and were highest for objects (2.31±2.21 s, n = 229), which was a significant difference compared with alive rat interactions (1.95±1.93 s, n = 3125, P = 0.002, U test; see Fig. 3.13C). Stuffed rat interactions were on average 2.27±2.59 s long (n = 176).

Overall, the data suggest that stuffed rats elicited the least interest, as compared to alive rats and objects. It can also be seen that objects were approached rapidly, and then sampled in a few long whisking periods. However, conclusions are complicated by uneven presentation times, and behavioral observations indicated that interest in objects was lost much more rapidly than in alive rat stimuli. This might not be reflected in the normalized interaction times (Fig. 3.13B) because of an early termination of object presentation in case of complete lack of interest by the subject rat, with an additional contribution of object novelty.

The interactions with the anesthetized rat were not quantified, mainly because this stimulus was presented on only three days. In addition, it differed from the other inanimate stimuli

in that it was hand-held by the experimenter and was sometimes moved towards the subject rat to attract its attention.

### 3.2.5 Influence of stimulus type on whisking parameters

In addition to differences in the level of interest, the whisking of subject rats onto stimulus rats was also substantially different from the touch of inanimate stimuli. The whisking onto both objects and the stuffed rat was much more regular than whisking onto stimulus rats, as can be seen by the peaks in whisking power in the 8-12 Hz frequency range in representative whisker traces (Fig. 3.14). Further characteristics of interactions with inanimate stimuli were higher whisking amplitudes and more protracted set angles, particularly for the stuffed rat as stimulus (Fig. 3.14C).

This was reflected on the population level, where whisking power and set angles differed between interactions with animate and inanimate stimuli. Whisking power during both object and stuffed rat touch was higher than during alive rat touch by a factor of ca. 2.5 (Fig. 3.15A), which was highly significant ($P<0.0001$ for both comparisons, $n = 200$ for alive rat interactions, $n = 20$ for objects, $n = 10$ for stuffed, U test). Whisking power was very similar during touch of the two types of inanimate stimuli ($P = 0.878$, U test). In the same way, the whisker set angle was also much lower during alive rat touch (mean set angle = 13.9±14.3°) than during object touch (24.1±12.6°) or stuffed rat touch (33.1±2.9°; see Fig. 3.15B). These differences were again highly significant for the comparison of alive rat touch with both stuffed rat touch ($P<0.0001$, U test) and object touch ($P = 0.002$, U test). The difference in set angles during stuffed rat and object touch was also significant ($P = 0.033$, U test).

In sum, the whiskers were moved with higher amplitude and regularity around a more protracted set point, when an inanimate stimulus was touched, and whisking was particularly stereotyped for stuffed rat touch. This was supported not only by the means, but also by the variances of the set angle distributions, which were lower for stuffed than alive rat touch ($P < 0.001$, Brown-Forsythe test). In contrast, set angles during object touch had only a slightly lower variance than those during alive rat touch, and the difference was not significant ($P = 0.499$, Brown-Forsythe test).

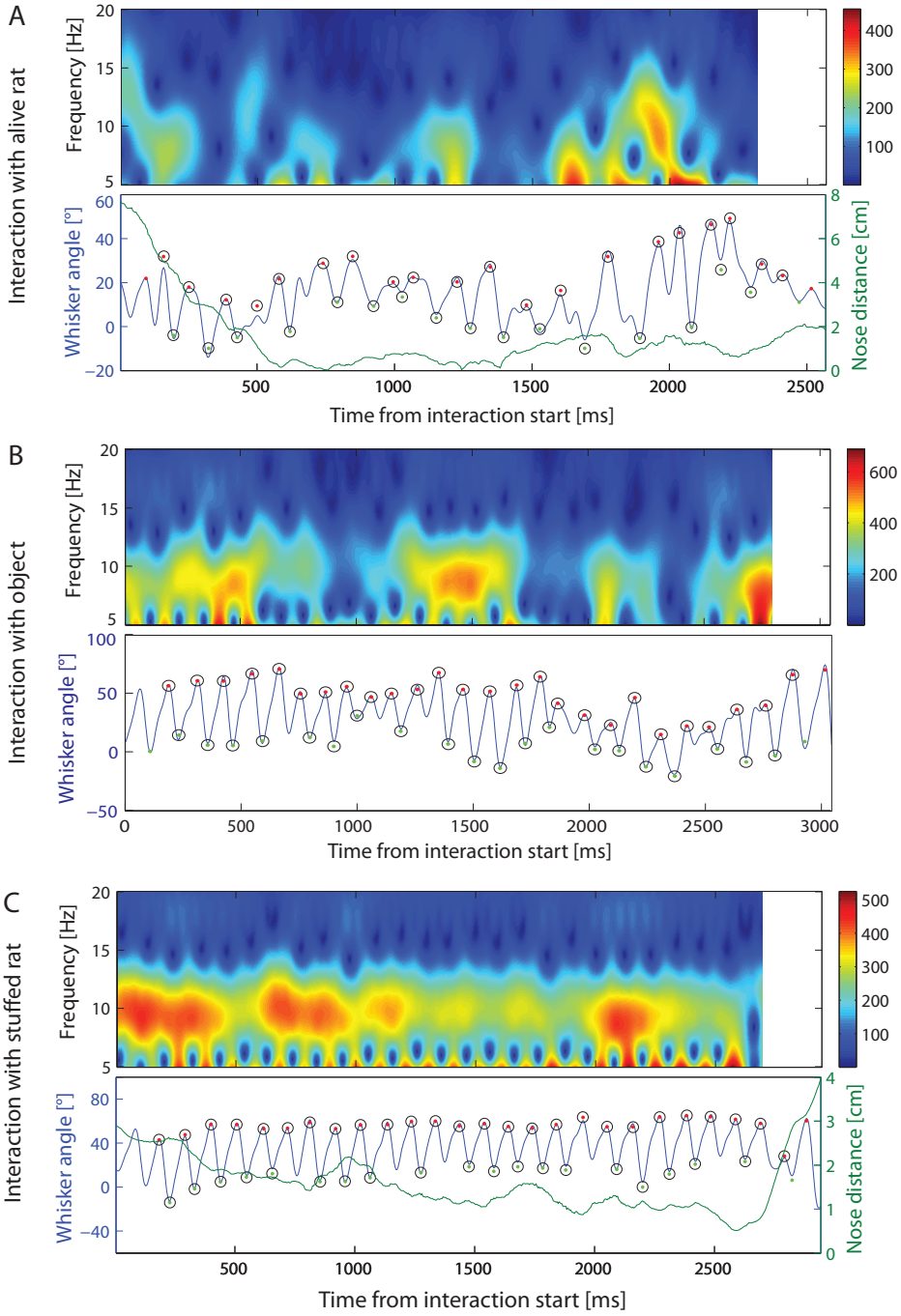



Figure 3.14: Example whisking traces of the subject rat onto different stimuli. A, Interaction with an alive rat. Power is low in the whisking frequency range (top), and the whisker is irregularly moving around a relatively retracted set point with mostly small-amplitude whisks (bottom). In the smoothed whisker trace in blue, the detected peaks and troughs are marked by red and green dots, respectively. Circles around these events indicate the period where an adjusted power threshold was crossed. The nose distance between the two rats is shown as a green line. B, Interaction with an object. The whisking is much more regular than in A, and there are power peaks around 10 Hz for prolonged periods. C, Interaction with a stuffed rat. Whisking is extremely regular with a peak in the 8-12 Hz frequency band power over the whole interaction (top). The whisker is also held more protracted than in A or B (bottom). The nose distance between stuffed and alive rat is shown as a green line. Note that scales of whisker angle, nose distance, and relative whisking power differ between panels.

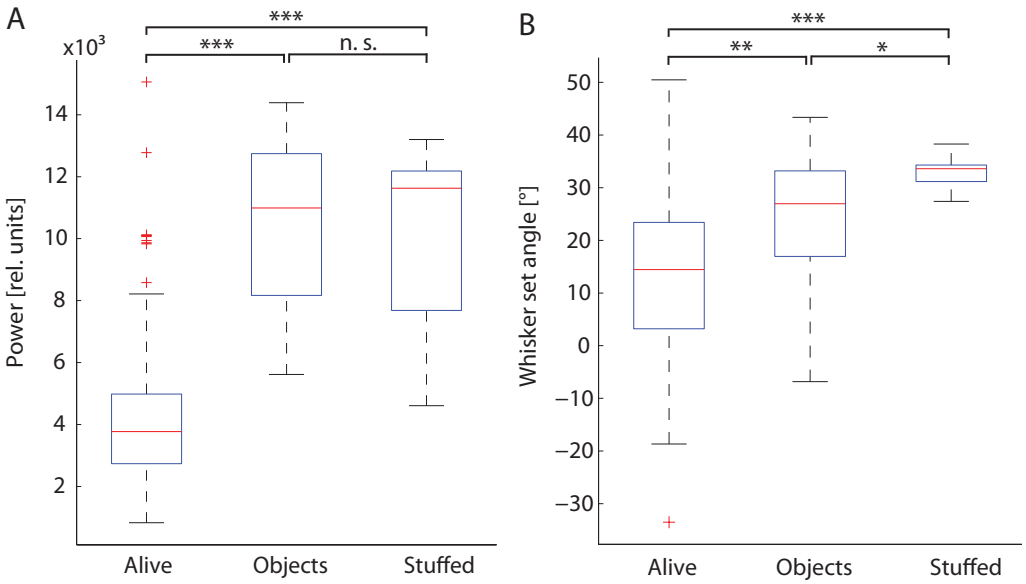



Figure 3.15: Whisking behavior during interactions with animate and inanimate stimuli. A, Whisking power was higher for object and stuffed rat touch than for the touch of alive conspecifics. B, Whisker set angles were higher during touch of inanimate stimuli, particularly for the stuffed rat. 'n.s.', not significant.

## 3.3 Physiology

### 3.3.1 Response modulations of single units during interactions

As this was the first study to examine responses of BC neurons during social touch, the first step was to observe responses on the level of individual units. In many units strong response increases during social touch were observed. The response increase of a representative RS from L5B is shown in Fig. 3.16A,B. This unit, for which the corresponding histological recording location is shown in Fig. 2.4, increased its firing rate from 1.03 Hz outside interactions (i.e., baseline) to 3.73 Hz during interactions ($P < 0.001$, permutation test). The response increase around interaction onset was also highly significant ($P = 0.002$, signed-rank test), when the firing rates in the 500 ms before and after the start of each interaction were compared. As shown in Fig. 3.16C, interactions were associated with decreasing whisking amplitudes and lower regularity.

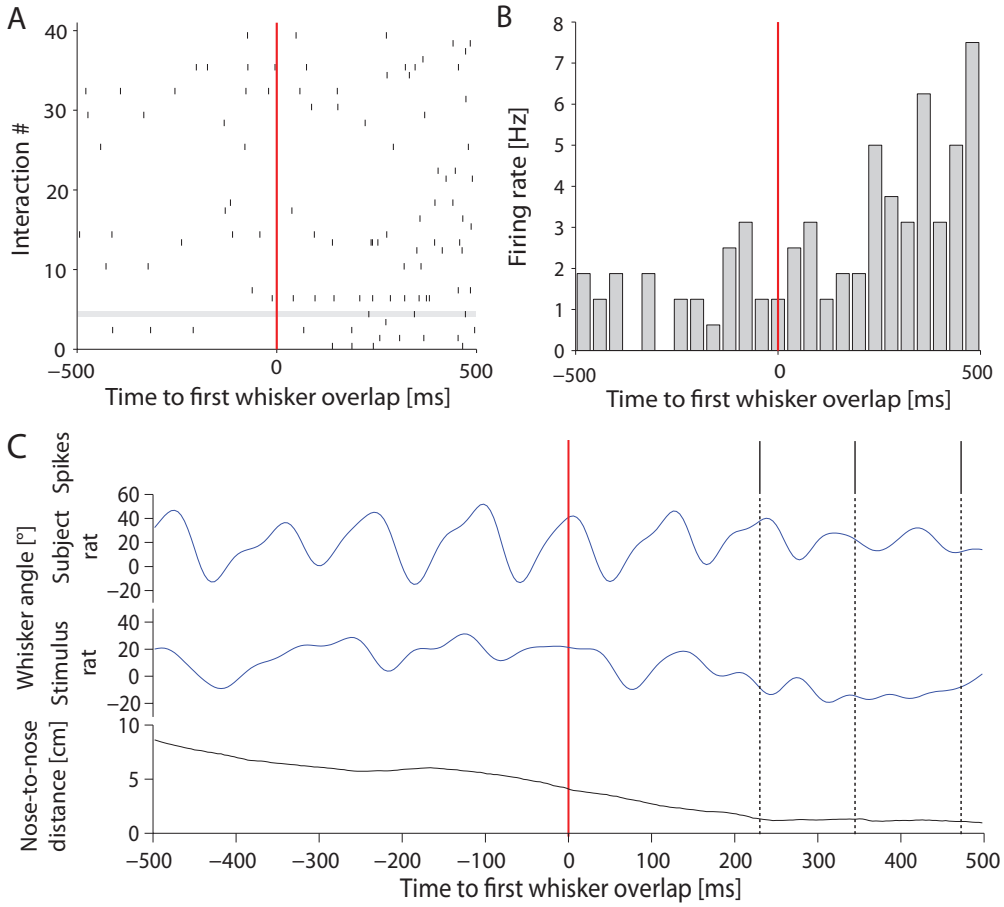



Figure 3.16: Response increase of a representative SU, a RS from L5B, at interaction onset. A, Raster plot of spikes in single interactions. Red line indicates the time of first whisker overlap. B, PSTH of responses shown in A. C, Whisker motion and nose-to-nose distance in an interaction (underlaid gray in A). Times of spikes are marked by black vertical lines (both solid and dashed). Note the decrease in amplitude and regularity after interaction start, in particular for the subject rat.

Responses typically started to appear after first whisker overlap, but for many units they peaked or plateaued only when the heads of the two rats touched for the first time (Fig. 3.17A, top left, and Fig. 3.17C, top left). At the end of interactions, response changes were often sharper (Fig. 3.17A, top right, and Fig. 3.17C, top right), in line with the observation that the time between the first whisker overlap and first head touch was much longer than the time between last head touch and last whisker overlap. In other words, rats approached each other slowly, but typically separated rapidly.

Observed responses in the neuronal population were very diverse, as can be seen in Fig. 3.17 and 3.19. Examples of strong response increases were observed in both RS (Fig. 3.16, Fig. 3.17C) and FS (Fig. 3.17A). The example FS increased its firing rate from 25.3 Hz at baseline to 37.1 Hz during interactions ($P < 0.001$, permutation test). Interestingly, the firing rate in the 500 ms before interaction start was much lower than baseline (17.0 Hz), and this was also highly significant ($P < 0.001$, permutation test). It has not been established, whether this lower firing rate before interactions was due to interaction-related inhibition, or was simply an effect of high firing rates during other periods of the baseline time, e.g. when the walls were touched. This was very improbable to happen within 500 ms before interaction onset, and could thus produce the appearance of inhibition.

While responses were easier to detect in highly active units, examples of units which were very sparsely activated by social touch were also observed. Thus, the unit shown in Fig. 3.17B, a L5B RS, had a baseline firing rate of only 0.12 Hz, which increased to 0.26 Hz during interactions. This response increase, as well as the increase at first head touch and decrease at interaction end, were significant ($P < 0.03$ for all three comparisons). The sparsely responding unit in Fig. 3.17B was recorded in the same session and at the same location as the one shown in Fig. 3.17C. This unit was also a RS which increased its response significantly ($P < 0.001$ for the three aforementioned comparisons). This serves to show that recordings from low-firing units could be achieved even in the presence of much more active units. The separation of the unit in Fig. 3.17B from the one in Fig. 3.17C, as well as others from this recording, is established with high probability, as the L-ratio of the former was 0.007. This placed it in the top eighth of all units by separation.

As shown above, there were strong responses of many units associated with social touch. However, it could be asked whether comparably strong responses were observed during the touch of other stimuli. This question has been investigated in depth for objects (see 3.3.9) and stuffed rats (see 3.3.10), while a quantification of wall and floor touch events, which did occur during baseline time, was not attempted. However, as Fig. 3.18 shows for an example unit, it was observed repeatedly that interactions were associated with peaks of activity which exceeded those dispersed over the baseline time. For the particular unit in Fig. 3.18 (same as shown in Fig. 3.56A,B), the 12 highest peaks were all associated with interactions, and

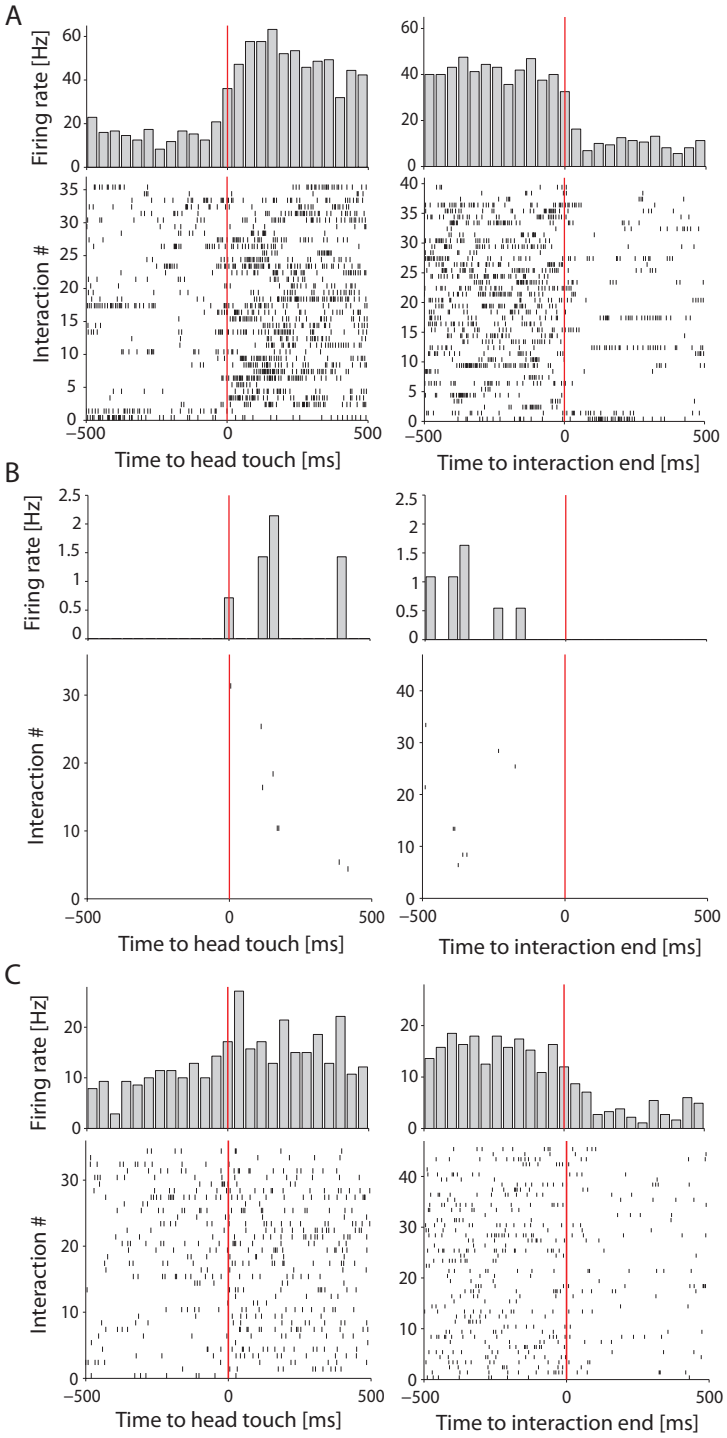



Figure 3.17: Example responses of SUs aligned to the time of first head touch (left column) and interaction end (right column). A, FS with a particularly strong response during interactions. The response sharply increases at the begin of head touch (response histogram in top left, raster plot of spikes in single interactions in bottom left). At interaction end, the response decreases even more sharply (right), indicating that in the end of interactions the head touch and whisker overlap events were much closer than at interaction start. B, Very sparse response increase of a RS during interactions. C, Another RS recorded in the same session and from the same tetrode as the unit shown in B. Note that the response increases before the head touch start event, but reaches the peak afterwards.

the peak firing rate during interactions was 14.6 Hz, as opposed to 6.2 Hz during baseline time. Amongst 41 distinctive peaks over 3.5 Hz, the firing rate during those associated with interactions (mean 6.8 Hz, n = 23) was significantly higher than for those independent of interactions (mean 4.4 Hz; n = 18, P = 0.004, U test).

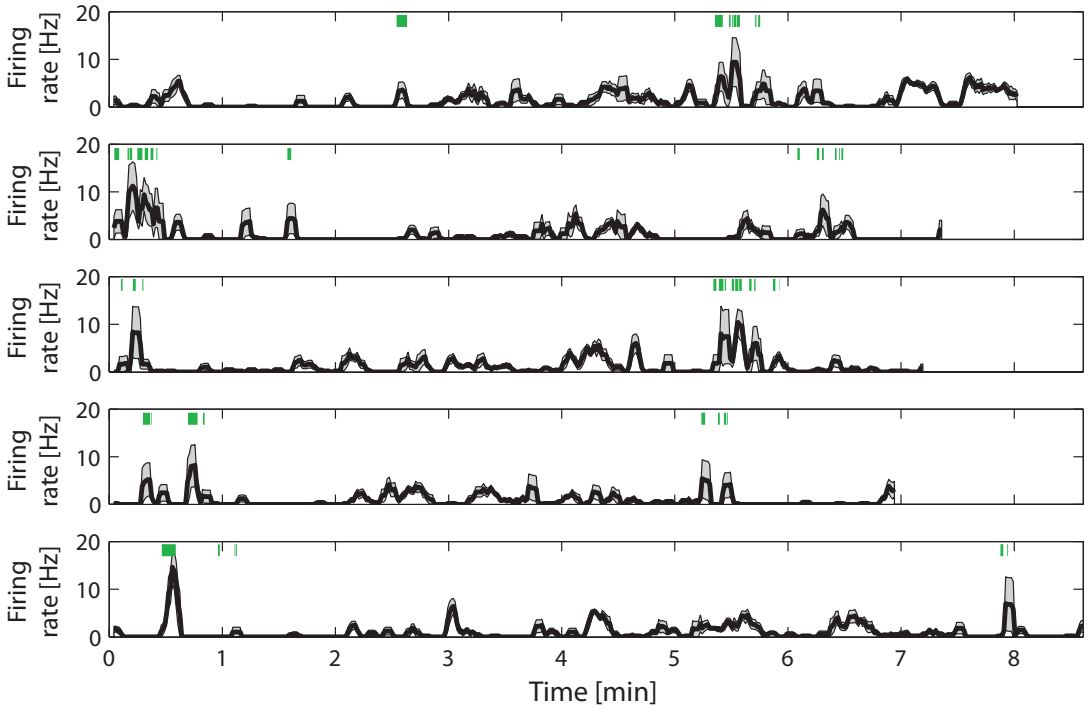



Figure 3.18: Moving average of the firing rate of a strongly modulated RS over all five stimulus rat presentation blocks of a recording session. Times of interactions are marked by green boxes. Note the strong response increases associated with interactions, and the absence of comparable peaks outside of interactions. It can also be seen that interactions were not evenly distributed, but rather occurred in bouts. For calculations of the moving average and the error bars, see 2.7.1. Same unit as shown in Fig. 3.56A,B.

A considerable diversity of responses was observed apart from response strengths and firing rates, as shown in Fig. 3.17. Thus, some units exhibited bursty firing, as displayed in Fig. 3.19A-D. These bursts were much higher in frequency than the oscillations in spike trains described in 3.3.2. Bursty units could be associated with both excitation (Fig. 3.19A) and inhibition (3.19C). Bursts with high intra-burst frequencies of 100 Hz and above were typically observed in FS, as the one shown in Fig. 3.19C,D.

While this study focuses on SUs, it should be noted that MUs often also showed very strong modulations by social touch. Thus, a MU recorded in L5A showed a sharp response increase at head touch (Fig. 3.19E). The ISI histogram (Fig. 3.19F) of this unit shows some of the criteria used for classification of clusters as MUs: there is no clear refractory time, indicated here by numerous spikes in the first bin of 2 ms width. In addition, the ISI histogram bin counts decreased smoothly, indicating that this was not a bursty FS.

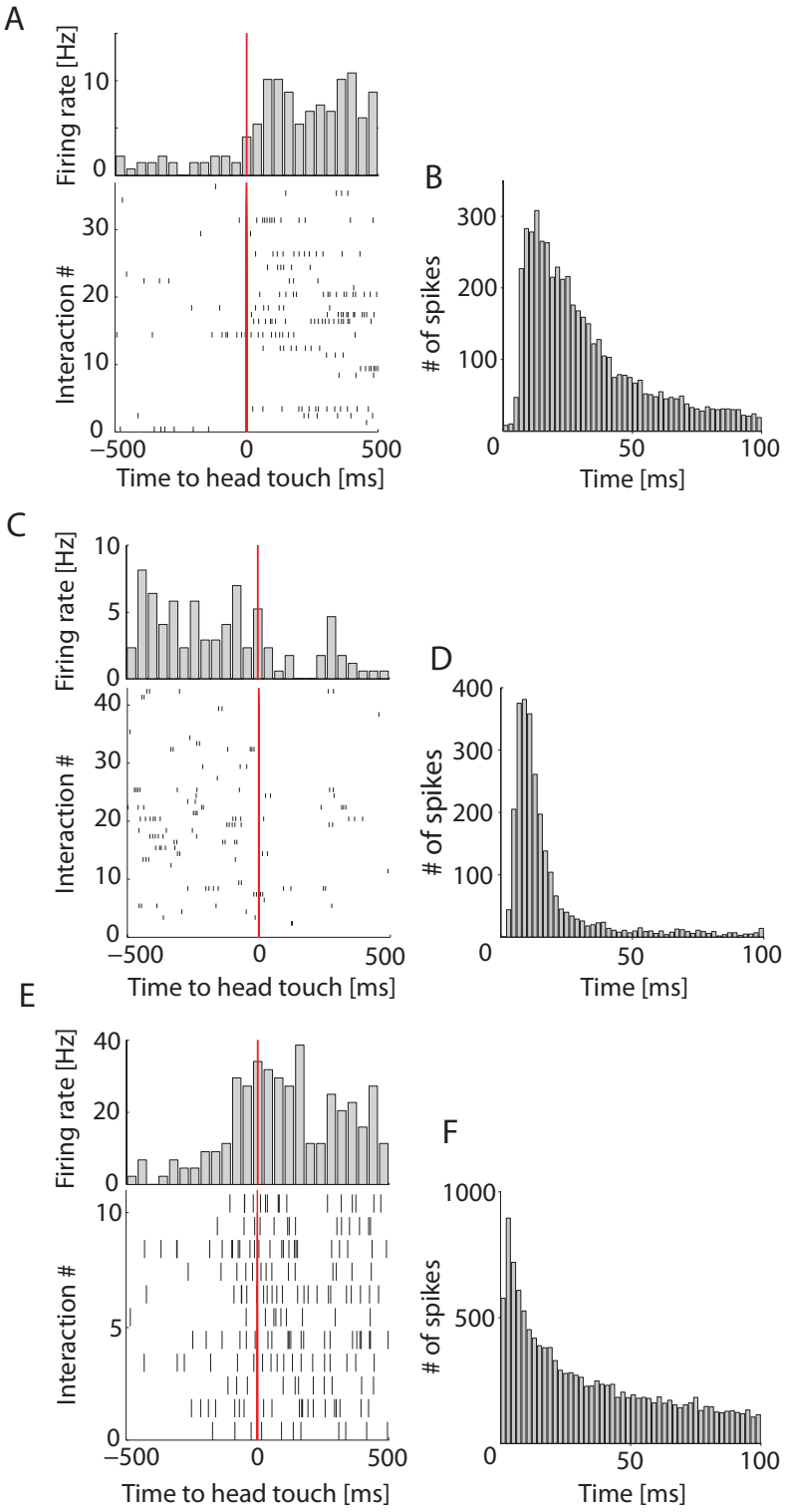



Figure 3.19: Diversity of neuronal responses in BC associated with social touch. A, Response histogram (top) and raster plot of spikes in single interactions (bottom) for a bursty RS. B, ISI histogram for the unit shown in A. Bursts had frequencies of up to 100 Hz. C, Same as A for a FS which decreased responses after head touch. D, Same as B for the unit shown in C. This unit fired high-frequency bursts with intra-burst frequencies of 80 to 125 Hz. E, Same as A for a MU. Responses increased sharply around the begin of head touch. F, Same as B for the unit shown in E. Note the lack of a refractory time and the smooth decrease of the ISI histogram, together indicative of a MU.

### 3.3.2 Oscillations in spike trains

**Examples of oscillatory firing** The spiking activity of SUs was oscillatory in many cases. The oscillation frequencies observed were most often in the range of theta frequencies (4-12 Hz), which includes the frequencies of whisker motion (6-12 Hz), or slightly above, i.e., in the lower beta range. The pattern of activity around interactions indicated that at least for some units, these oscillations were associated with whisking. One example RS from L5B showed very regular activity with a peak frequency of 8.7 Hz (Fig. 3.20A-C). This example cell increased its firing rate from 4.60 Hz during baseline time to 7.26 Hz during interactions (Fig. 3.20D), which was significant ($P<0.001$, permutation test). There was, however, a peculiar and seemingly contradictory pattern of activity change around interaction onsets: the activity did not increase around the onset of interactions, i.e., first whisker overlap (Fig. 3.20A; $P = 0.915$, $n = 38$, signed-rank test), and then decreased highly significantly after head touch (Fig. 3.20B; $P <0.001$, $n = 32$, signed-rank test). Correspondingly, firing increased at the end of interactions, which was again significant (data not shown; $P = 0.034$, $n = 38$, signed-rank test). The apparent contradiction between an overall response increase in interactions, as compared to baseline, and the response decrease at actual interaction start and end, was explained by a particularly high firing rate immediately before and after interactions. Thus, the firing rate in the 500 ms before whisker overlap start was 8.84 Hz (Fig. 3.20D), which was a highly significant increase compared to the firing rate during baseline time ($P<0.001$, permutation test). It was also higher than the interacton firing rate, although this difference was not significant ($P = 0.180$, $n = 38$, signed-rank test).

Oscillations were, however, present at different frequencies, and the units showed different patterns of activity around interaction onset. Fig. 3.21 presents further examples of oscillations in spike trains. The cell shown in Fig. 3.21A-C, a L5B RS, had its peak oscillatory frequency at 18.2 Hz (Fig. 3.21B). Similar to the unit shown in 3.20, it also decreased activity after head touch (Fig. 3.21A), and fired at higher rates immediately before interaction onset than during interactions (Fig. 3.21C), a significant decrease from 13.26 Hz during the 'pre-time' to 10.23 Hz in interactions ($P < 0.001$, signed-rank test). However, this unit's activity was lower during interactions than even in the baseline time (11.43 Hz), and this decrease was also significant ($P = 0.003$, permutation test).

A third example unit, also a RS shown in Fig. 3.21D-F, had an oscillatory frequency peak at 15.4 Hz (Fig. 3.21E), and thus between the two aforementioned units. While the frequency of oscillations was much higher than for the example cell in Fig. 3.20, the pattern of rate change around interactions observed here was similar. This unit also decreased activity around the time of head touch (Fig. 3.21D; $P = 0.003$, $n = 40$, signed-rank test), and showed an overall firing rate ranking of 'pre-time' (10.40 Hz) > interaction time (9.74 Hz) > baseline (7.40 Hz).

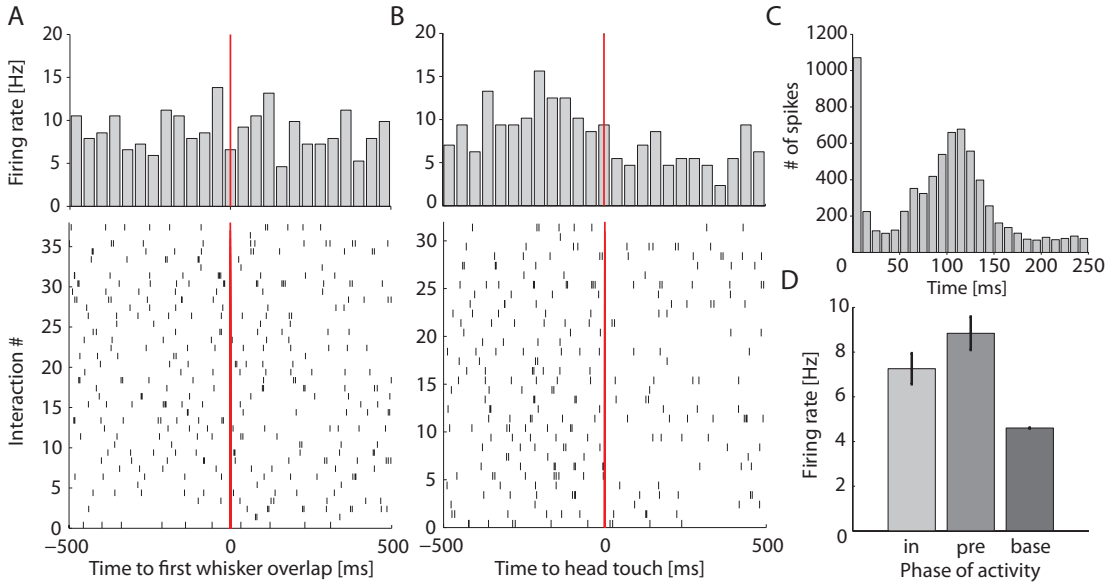



Figure 3.20: Example unit showing strong oscillatory activity. A, The firing of this unit was very regular (raster plot of spikes in single interactions at the bottom), and activity did not change after first whisker overlap (response histogram at the top). B, After head touch, the firing rate of this unit strongly decreased. C, The ISI histogram shows a peak at 8.7 Hz. Note that the abscissa scaling is different than in Fig. 3.19B,D,F. D, The firing rate during interactions ('in') was higher than during baseline time ('base'), but lower than during the 500 ms preceding first whisker overlap ('pre'). Error bars are standard errors of the mean.

**Comparison of regular-spiker and fast-spiker oscillatory activity** The three example units shown in Fig. 3.20 and Fig. 3.21 were all RS, and also on the population level it was observed that RS had a much stronger tendency to fire regularly in a wide range of frequencies (Fig. 3.22). As a measure of oscillation strength, the normalized oscillatory power was calculated by dividing the maximum power value within a window of 3 Hz width by the mean oscillatory power over all analyzed frequencies (3-49 Hz). The sliding windows were set such that window centers were between 4.5 and 47.5 Hz, and these windows were shifted in steps of 1 Hz. The normalized oscillatory power was significantly higher for RS than FS in the range of 10.5 to 27.5 Hz, and the significance level for each comparison was below 0.001 in the range of 12.5 to 25.5 Hz (for RS n = 250, for FS n = 81, U test).

In the lowest frequency range analyzed (4.5-6.5 Hz), the pattern was reversed, and FS showed higher oscillatory power than RS (Fig. 3.23). This indicates that the effects observed here are not artifacts of a generally higher firing rate of FS, in conjunction with the normalization procedure, as discussed in 4.3.7.

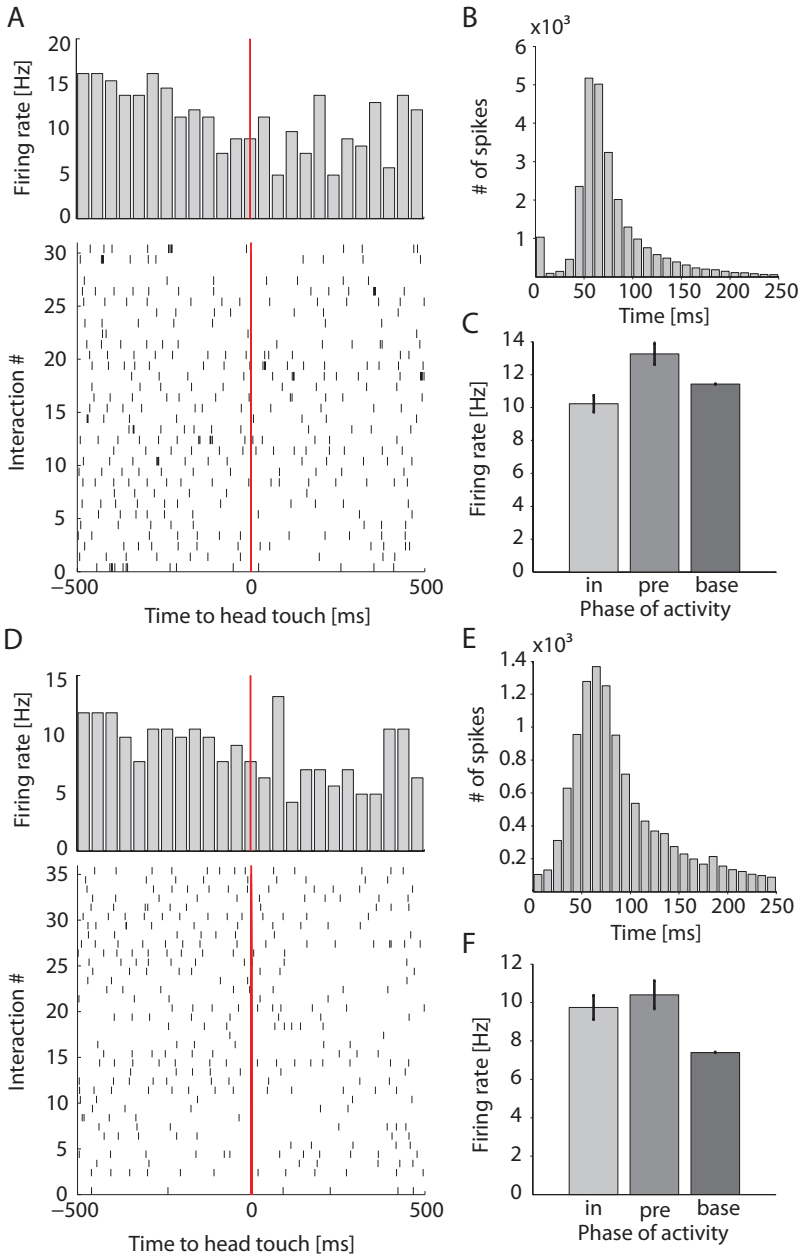



Figure 3.21: Units showed diverse oscillatory frequencies and different patterns of rate change during interactions and the time immediately preceding them. A, Response histogram of an example unit (top) and raster plot of spikes in single interactions (bottom). Similar to the unit shown in 3.20, activity decreased after head touch. B, ISI histogram for the unit shown in A. Peak oscillatory frequency was 18.2 Hz. C, Firing rates during different behavioral phases for the unit shown in A. 'In' indicates 'in interactions', 'pre' indicates 'in the 500 ms period before first whisker overlap'. This unit was inhibited during interactions as compared to baseline. Error bars indicate standard errors of the mean. D-F, Same as A-C for an example unit with peak oscillatory frequency of 15.4 Hz. As for the unit shown in 3.20, activity was increased during interactions, but highest immediately before their start.

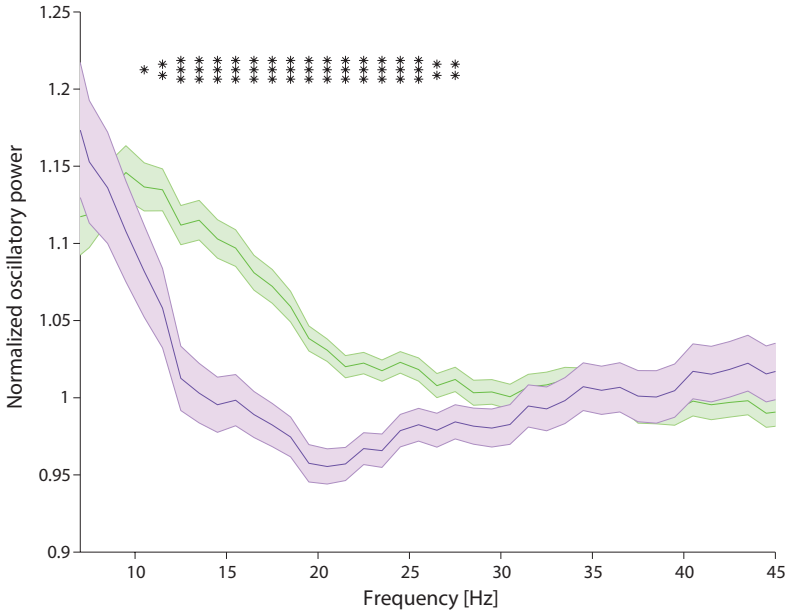



Figure 3.22: Comparison of the power of oscillations in the spike trains of RS and FS at different frequencies. RS are marked in green and FS in violet. Error margins are standard errors of the mean. Asterisks indicate significance values, with the asterisks corresponding to one comparison being grouped below each other.

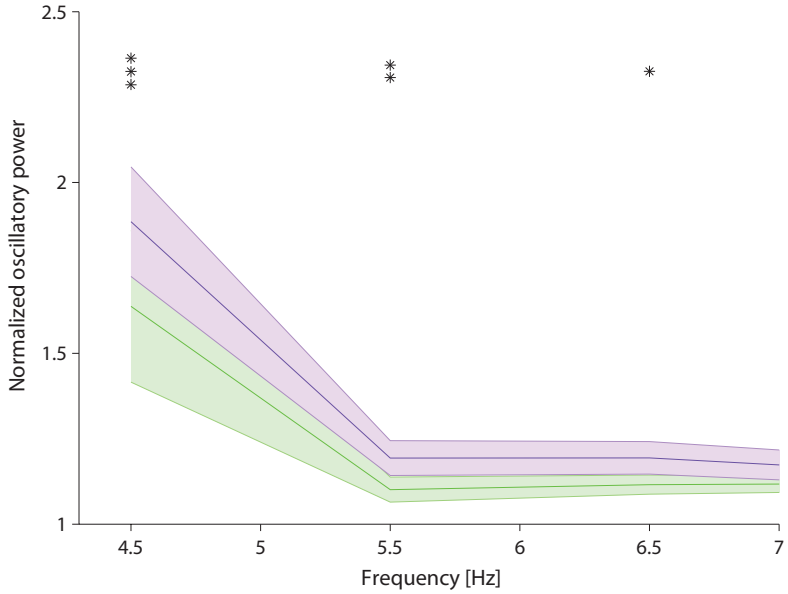



Figure 3.23: Comparison of the power of oscillations in the spike trains of RS and FS for the lowest frequencies analyzed. RS are marked in green and FS in violet. Error margins are standard errors of the mean. Asterisks indicate significance values, with the asterisks corresponding to one comparison being grouped below each other.

### 3.3.3 Overall modulation of the neuronal population during social interactions

**Regular-spikers** Many units displayed behavior similar to the ones shown in Fig. 3.16 and Fig. 3.17, and, as a consequence, the neuronal population recorded exhibited an overall change of activity during social interactions. As Fig. 3.24 shows, RS in BC on average increased their response at interaction onset (Fig. 3.24A), and this elevated activity was maintained until the end of interactions, when the firing rate fell back to baseline (Fig. 3.24B). Firing rates of RS increased from a baseline of 2.78±3.76 Hz (median 1.21 Hz) to 3.56±4.80 Hz (median 1.42 Hz) during interactions. This firing rate increase was highly significant ($P < 0.0001$, $n = 242$, signed-rank test). The population PSTH of MU responses at interaction onset can be found as the responses of untrimmed animals in Fig. 3.46D.

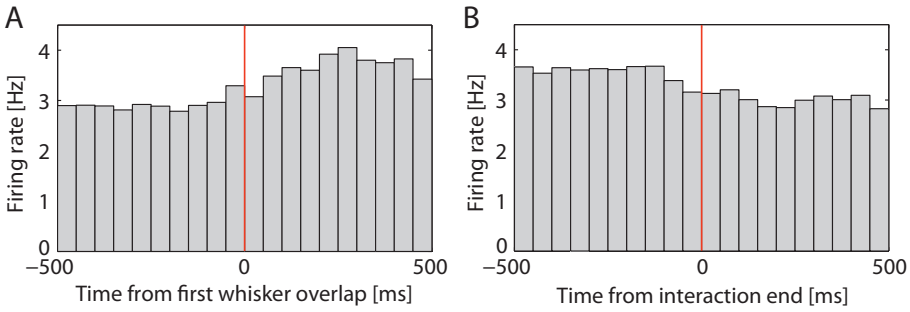



Figure 3.24: A, PSTH of mean RS activity around interaction onset. There is a consistent increase in activity, which reaches its peak ca. 250 ms after first whisker overlap. B, PSTH of mean RS activity around interaction offset.

Figure 3.25 details the response changes during interactions for each RS, and indicates the significance levels of these modulations. Out of 242 RS, 93 (38.4%) were significantly modulated during interactions at a significance level of 0.05, as determined by the permutation test, 70 units (28.9%) reached P-values ≤0.01, and 53 (21.9%) reached P-values ≤0.001. Although there was an overall firing increase, there was a large number of units which decreased responses during interactions (101 of 242), apparently indicating a high prevalence of inhibition. Response decreases were, however, more common in low-firing units, as shown by the positive correlation between baseline firing rate and the in vs. out firing rate difference (i.e., interaction firing rate minus baseline; $R = 0.261$, $P < 0.001$, Pearson's correlation). Inhibitory responses were, in addition, weaker than excitatory responses (median increase 0.53 Hz, median decrease 0.24 Hz). Finally, inhibitory responses were much more rarely significant (20 of 101, or 19.8% for inhibition, but 73 of 141, or 51.8% for excitation, at a significance level of 0.05). All this points in the direction that of those units which were found to decrease activity, many were not inhibited, but rather not modulated by social touch, and their

apparent response decrease was due to independent fluctuations. However, some instances of evident inhibition were also observed (Fig. 3.19C).

Responses were more often significant for high-firing units, and in particular the units with highly significant modulations ($P \leq 0.001$) fired at rates above average (Fig. 3.25B). At the same time, there was a highly significant negative correlation of the absolute value of response indices with baseline firing rates ($R = -0.326$, $P < 0.0001$, $n = 240$, Pearson's correlation). This indicates that although modulations were found to be significant more often for high-firing units, actual modulation strength of low-firing units was higher. Higher noise might have contributed to the higher modulation observed in low-firing units. Nevertheless, the strong modulation of low-firing units suggests that a low firing rate of a unit does not preclude a strong functional modulation. By extension, this supports the view that 38.4% is a lower-bound estimate for the number of RS modulated by social touch. Presumably, the modulation of many neurons with low firing rates did not reach significance because statistical power for low numbers of spikes was insufficient, especially if the number of interactions on a particular day was also low.

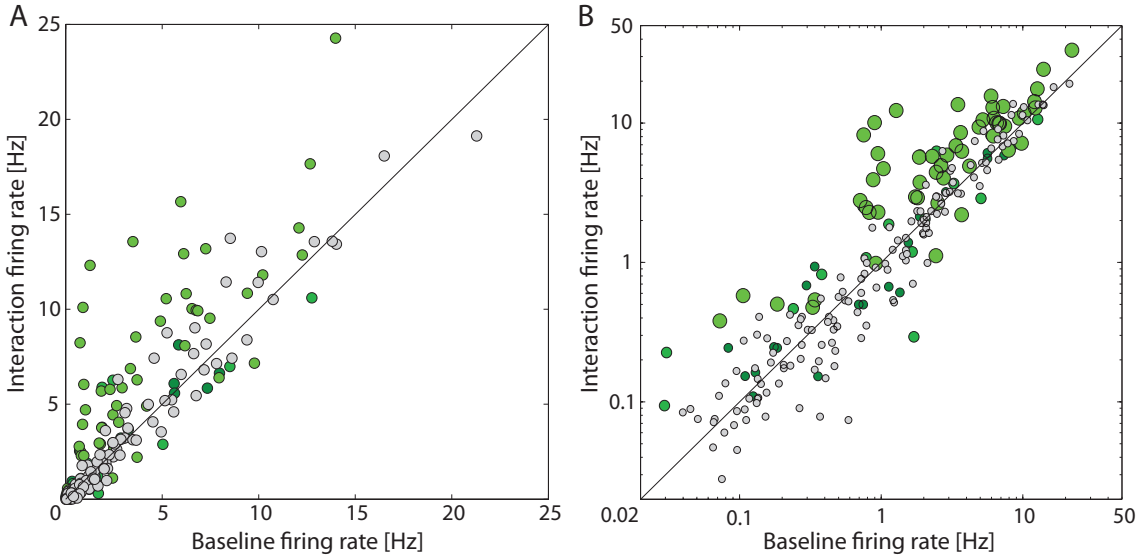



Figure 3.25: Response modulations of RS during social interactions, highlighting the significantly modulated units. A, Many units strongly increased firing rates during interactions, but significant inhibition was also observed. Color indicates significance level of modulation: grey - not significant, dark/medium/light green - significant at alpha level of 0.05, 0.01, and 0.001, respectively. One unit firing at a rate of 35 Hz during interactions was omitted. B, Same data as in A, plotted logarithmically. Inhibition was more common in low-firing units, while the probability to observe a significant firing modulation was higher in highly active units. Color code as in A; additionally, the three significance levels are coded in the size of markers, the largest being the most significant.

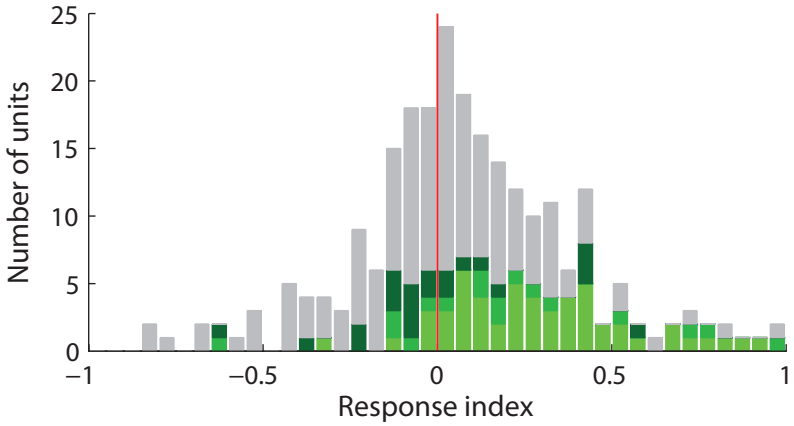



Figure 3.26: Distribution of RS response indices.On the population level, there is a strong shift towards positive indices, i.e., excitation. Color indicates significance level of modulation: grey - not significant, dark/medium/light green - significant at alpha level of 0.05, 0.01, and 0.001, respectively.

As a measure of response modulation independent of firing rate, a response index (see 2.7.2) was calculated. As Fig. 3.26 shows, this index was also strongly shifted towards positive values, indicating excitation on the population level. The average index was 0.080±0.319, and the median was 0.055, which was a highly significant shift from zero ($P < 0.0001$, $n = 240$, one-sample signed-rank test). The number of units for which indices are available is lower than the overall number of recorded RS, as two units had no spikes during both interactions and shifted baseline time, and a response index could not be calculated in this case.

**Fast-spikers** Similar to RS, FS displayed consistent response increases during interactions, as shown in Fig. 3.27. The firing rate increased during interactions from 6.89±9.75 Hz (median 2.64 Hz) to 8.83±11.97 Hz (median 3.39 Hz). This response increase was highly significant ($P = 0.0002$, $n = 72$, signed-rank test). While the response increase of FS around interaction onset (Fig. 3.27A) is very similar to the corresponding PSTH for RS (Fig. 3.24A), the responses of FS started to decrease 300-400 ms before interaction offset (Fig. 3.27B), while the response decrease of RS at the end of interactions was rather abrupt (3.24B).

The percentage of FS which were significantly modulated by social touch was 47.2%, and thus higher than for RS (38.4%). Figure 3.28 shows the distribution of interaction-related response modulations and their significance level for individual FS. As for RS, significant modulations were more often observed for highly active units, which might explain the higher percentage of such units amongst FS. Also similarly to RS, inhibitory responses of FS were much more rarely significant than excitatory responses (5 of 22, or 22.7% for inhibition, but 29 of 50, or 58.0% for excitation, at a significance level of 0.05). Although responses of FS were thus comparable to RS, strong modulations were more rare for FS than RS. This is visible in the larger deviation of responses from unity line in Fig. 3.25B, as compared to Fig. 3.28B,

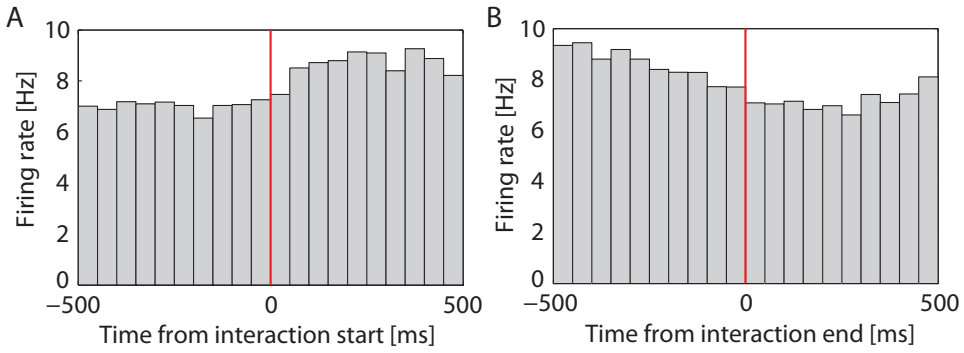



Figure 3.27: A, PSTH of mean FS activity around interaction onset. There is a consistent increase in activity, very similar to the population response of RS (Fig. 3.24A). B, PSTH of mean FS activity around interaction offset. The response begins to decrease 300-400 ms before interaction offset, in contrast to the response of RS (Fig. 3.24B)

as well as in Fig. 3.29, which shows the distribution of response indices for FS. It can be seen that response indices over 0.5, which correspond to an over threefold increase, were rare in FS, and similarly negative indices were not observed at all. In other words, the variance of the index distribution was higher for RS (0.102) than FS (0.061), and this difference was significant ($P = 0.050$, Brown-Forsythe test). This was also reflected by the fact that the mean absolute value of the index, which quantifies response modulation independently of its sign, was higher in RS ($0.242 \pm 0.222$) than in FS ($0.210 \pm 0.192$).

Mean response indices were $0.142 \pm 0.247$ for FS (median = 0.117), which was a highly significant shift from zero ($P < 0.0001$, $n = 72$, one-sample signed-rank test). While indices were higher for FS than RS (mean = 0.080), this difference was not significant ($P = 0.111$, $n = 72$ for FS and $n = 240$ for RS, U test).

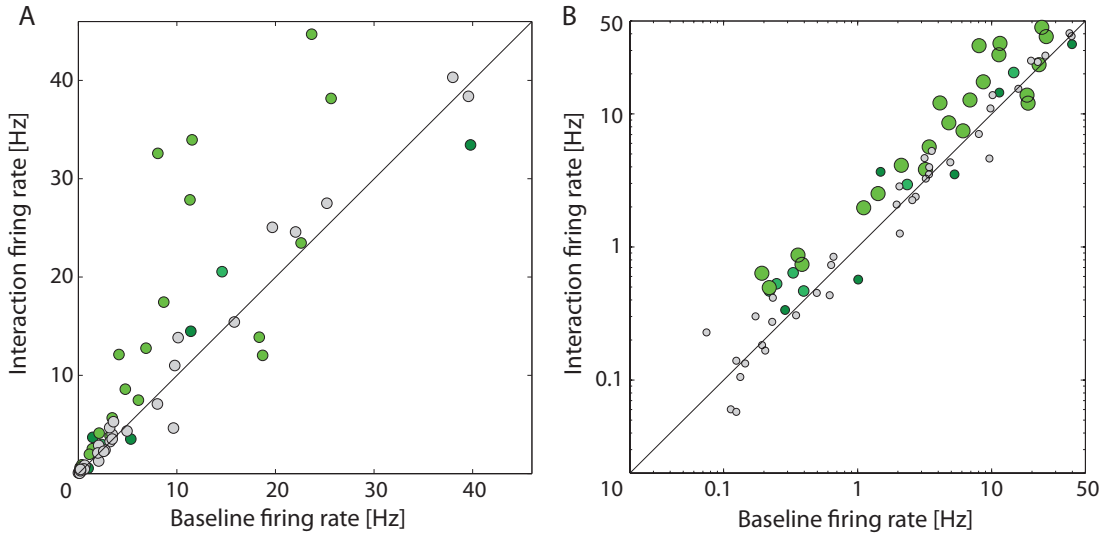



Figure 3.28: Response modulations of FS during social interactions, highlighting the significantly modulated units. A, The majority of units increased firing rates during interactions, but strongly modulated units were less common than amongst RS (Fig. 3.25). Color indicates significance level of modulation: grey - not significant, dark/medium/light green - significant at alpha level of 0.05, 0.01, and 0.001, respectively. B, Same data as in A, plotted logarithmically. The probability to observe a significant firing modulation was higher in highly active neurons. Color code as in A; additionally, the three significance levels are coded in the size of markers, the largest being the most significant.

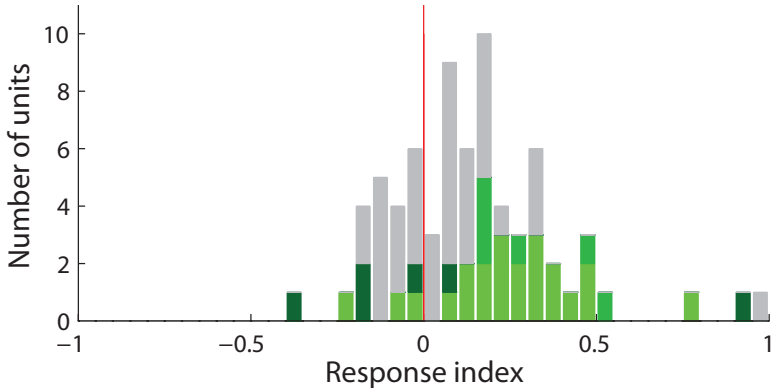



Figure 3.29: Distribution of FS response indices. On the population level, there is a strong shift towards positive indices, i.e., excitation. Color indicates significance level of modulation: grey - not significant, dark/medium/light green - significant at alpha level of 0.05, 0.01, and 0.001, respectively. Strong modulation, both excitatory and inhibitory, is less common than in RS (Fig. 3.26)

### 3.3.4 Firing rate and response variations over layers

Histology allowed the assignment of the majority of SUs to cortical layers. As discussed in 4.3.2, no L1 SUs were recorded, and thus this analysis is restricted to five layers: L2/3, which is not further distinguished in BC by most authors, L4, L5A, L5B, and L6. This analysis has been performed for RS and FS separately.

**Regular-spiker baseline firing rates** Baseline firing rates of RS (Fig. 3.30A) were homogenously low in L2/3, and both higher and more diverse in L4. Baseline firing rates of RS were highest in L5A and L5B. L5A RS fired at 2.77 Hz on average, and L5B RS fired at 3.03 Hz. Baseline firing rates in L6 were comparable to those in L4, but seemed to be less variable. However, only six RS were recorded in L6. As indicated in Fig. 3.30A, some of these rate differences were highly significant. In particular, firing rates for L2/3 were significantly lower than for L5A ($P < 0.001$, U test), L5B ($P < 0.001$), and L6 ($P = 0.003$). The difference of L2/3 to L4 also came close to significance ($P = 0.073$). In addition, L5B RS fired at significantly higher rates than L4 RS ($P = 0.044$). For means, standard deviations, and medians of RS baseline firing rates, see Table 1.

Table 1: Mean and median baseline firing rates of RS and FS. In addition, the ratio of FS to RS median firing rates and the number of entries for each cell type are given.

| | Mean RS baseline rate [Hz] | Median RS baseline rate [Hz] | n (RS) | Mean FS baseline rate [Hz] | Median FS baseline rate [Hz] | n (FS) | FS/RS rate ratio |
|---|---|---|---|---|---|---|---|
| L2/3 | 0.22±0.19 | 0.14 | 23 | 2.62±7.40 | 0.29 | 10 | 2.07 |
| L4 | 2.08±3.4 | 0.42 | 25 | 0.45±0.50 | 0.23 | 16 | 0.55 |
| L5A | 2.77±3.66 | 1.20 | 32 | 1.61±1.35 | 1.96 | 5 | 1.63 |
| L5B | 3.03±3.96 | 1.50 | 65 | 10.78±10.11 | 8.35 | 16 | 5.57 |
| L6 | 1.79±1.41 | 1.79 | 6 | 21.21±14.80 | 22.58 | 9 | 12.61 |

**Fast-spiker baseline firing rates** Differences between FS recorded in different layers were even more pronounced (Fig. 3.30B), so that even for the smaller sample of FS a clear picture emerged. Baseline firing rates of FS were again homogenously low in L2/3 (median 0.29), except for one outlier, which increased the mean to 2.62 Hz. The FS from L4 had the lowest mean firing rates (0.45 Hz), which were in fact much lower than those of L4 RS. In contrast to RS, where L5A and L5B firing rates were comparable, for FS there was a clear distinction, where L5A FS fired at low rates (mean 1.61 Hz), while L5B FS had very high baseline rates (10.78 Hz). Layer 6 FS had extremely high baseline firing rates of 21.21 Hz on average. Although the number of units per layer was relatively low, in particular for L5A, all comparisons between L2/3, L4, and L5A on the one hand, and both L5B and L6 on the other hand, were statistically significant (Fig. 3.30B). For means, standard deviations, and medians of FS baseline firing rates see Table 1.

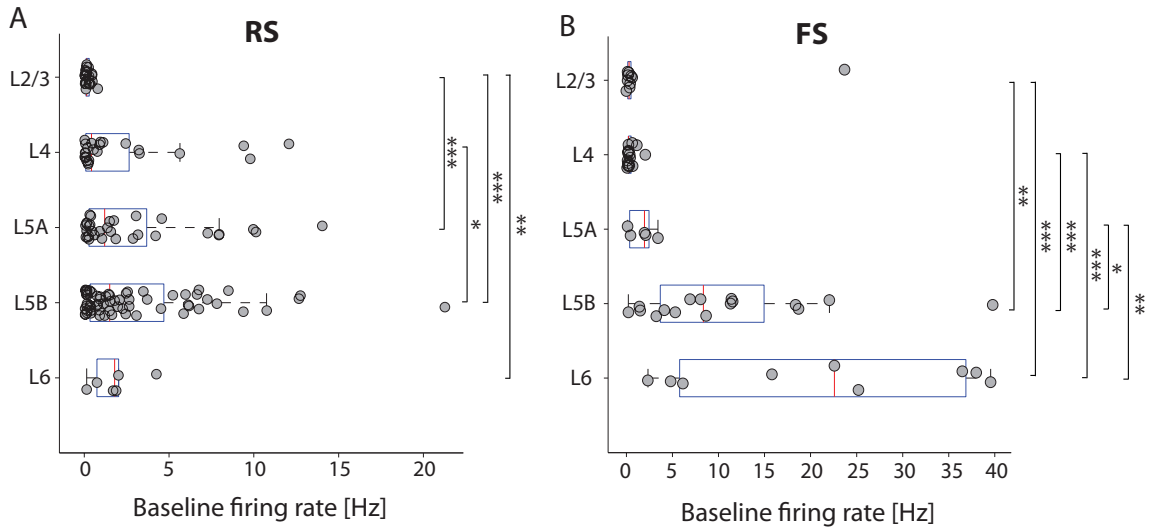



Figure 3.30: Baseline firing rates of SUs recorded in different layers. A, Boxplot of the baseline rates of RS recorded in different layers, overlaid with a scatterplot of the baseline rates of these units. Statistical significance of baseline rate comparisons between layers is indicated by asterisks on the right side. B, Box-scatter plot of the baseline rates of FS. Conventions as in A.

The overall higher firing rates observed in FS compared to RS (Fig. 3.4) were thus not due to FS from all layers in equal measure (see Table 1). As pointed out earlier, L4 was unique in that FS had lower firing rates than RS. In other layers, FS fired at higher rates, but this was much more pronounced in L5B and L6 than in L2/3 or L5A. The firing rate differences between FS and RS were only significant for L5B and L6 ($P < 0.001$ for both comparisons, U test).

**Regular-spiker responses during interactions** The distribution of RS firing rates during interactions (Fig. 3.31) generally matches the baseline rates shown in Fig. 3.30A. Layer 5B RS were also the most active during interactions, and L2/3 RS were particularly silent. For means, standard deviations, and medians of RS firing rates during interactions, see Table 2.

Although the coarse patterns of firing rate observed in different layers were similar for interaction and baseline periods, modulations during interactions were layer-dependent. While this information is implicit in Figs. 3.30A and 3.31, this becomes much clearer when either response indices are calculated, or the response increases are compared in population PSTHs constructed from neuronal activity around the onset of interactions. As can be seen in Fig. 3.32, L5B contributed most spikes per cell to the average response increase. Layer 2/3 also

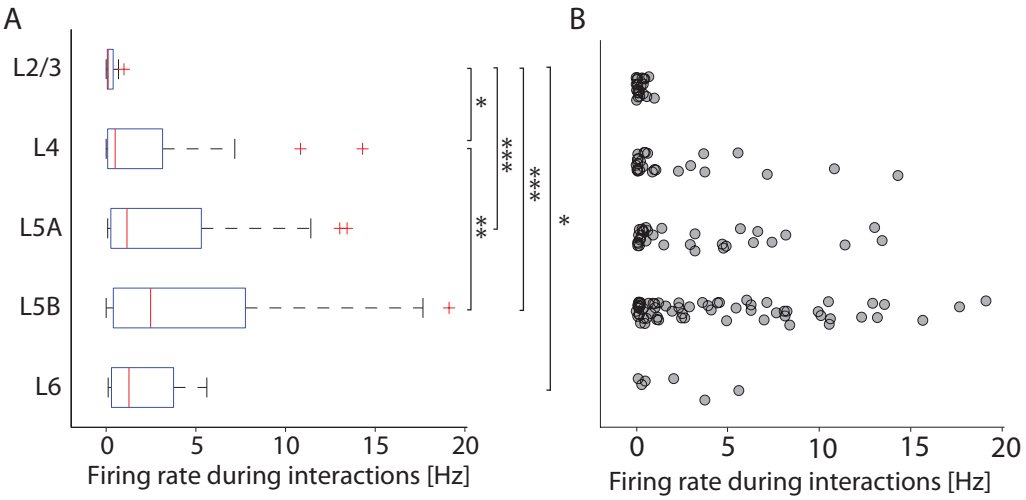



Figure 3.31: Firing rates of RS during interactions as a function of cortical layer. A, In line with the baseline firing rates, evoked firing was also highest in L5B, and weakest in L2/3. Statistical significance of evoked firing rate comparisons between layers is indicated by asterisks on the right side. B, Scatterplot of the responses shown in A. While all layers contained RS with very low firing rates, the distribution of highly active RS was skewed much in favor of L5B, and in particular against L2/3, where they were completely absent.

Table 2: Mean and median interaction firing rates of RS and FS. In addition, the ratio of FS to RS median firing rates is given.

| | Mean RS interaction rate [Hz] | Median RS interaction rate [Hz] | Mean FS interaction rate [Hz] | Median FS interaction rate [Hz] | FS/RS rate ratio |
|---|---|---|---|---|---|
| L2/3 | 0.24±0.25 | 0.11 | 4.77±14.04 | 0.44 | 4.00 |
| L4 | 2.25±3.67 | 0.50 | 0.61±0.48 | 0.48 | 0.96 |
| L5A | 3.25±3.96 | 1.15 | 2.41±2.46 | 2.08 | 1.81 |
| L5B | 4.50±4.93 | 2.48 | 15.51±11.73 | 13.30 | 5.36 |
| L6 | 2.05±2.23 | 1.27 | 22.06±14.09 | 23.45 | 18.46 |

clearly increased responses after interaction onset, but at a much lower firing rate level. Layer 5A and L6 did not consistently increase acitivity in the 500 ms after interaction onset. The response was expected to be particularly strong in L4 as the main cortical input layer. This was not observed, although a distinction can be made between L4 RS recorded in a medio-anterior location comparable to other RS recordings, and a group of L4 RS which were recorded in a more latero-posterior location than the rest of RS. This subset, which has already been highlighted in Fig. 3.7, did not show response increases after interaction onset, while the other L4 RS did (Fig. 3.32, dashed vs. solid brown lines). Although these units seem to have had somewhat distinct response properties, as both were histologically shown to be located in BC, they have been pooled in all other analyses.

As the different firing rates of RS from different layers might mask the magnitude of response modulation in Fig. 3.32, responses were additionally plotted after normalization to

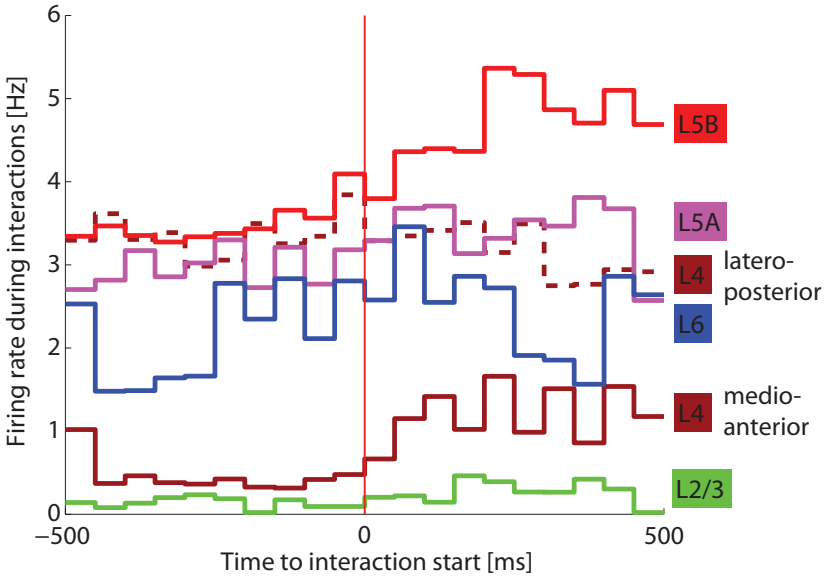



Figure 3.32: Population PSTH of responses around interaction onsets for RS, split by cortical layer. Units from L4 are distinguished by recording location within BC, with the medio-anterior location (solid brown line) corresponding to the location where recordings from other layers were conducted.

firing rate (Fig. 3.36A). It can be seen that L5B responses increased most consistently, but L2/3 responses also increased with even larger relative magnitude. The course of averaged response increases in L2/3 is much less smooth, however, presumably due to far lower firing rates and related higher noise in L2/3 RS firing.

The measure used to quantify modulation over the course of complete interactions were response indices (see 2.7.2). These indices, which ranged between +1 for very strong excitation and -1 for very strong inhibition, were significantly different between layers ($P = 0.041$, Kruskal-Wallis test), showing that, as already indicated by population PSTHs, response properties of RS were dependent on the layer (Fig. 3.33). Importantly, L5B RS not only fired more than RS from other layers, but also had the highest response indices (mean $\approx 0.2$). When considering these responses, it should be kept in mind that an index of 0.2 already corresponds to a 50% response increase. Response indices were around zero for L2/3, indicating that the response increases observed in Fig. 3.32 were transient. For L6 RS, response indices were also relatively high (mean $\approx 0.1$), for L4 they were weaker, but consistenly positive, and for L5A they were close to zero. For means, standard deviations, and medians of RS response indices, see Table 3.

**Fast-spiker responses during interactions** In line with the baseline rate differences shown in Fig. 3.30B, the population PSTH of FS responses around interaction onset (Fig.

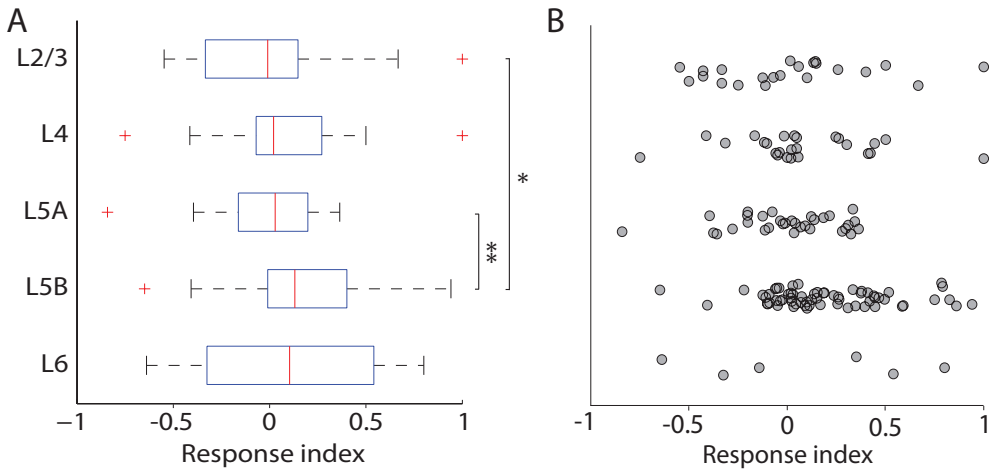



Figure 3.33: Response indices of RS as a function of cortical layer. A, Indices were highest in L5B, and lowest in L2/3. Statistical significance of index comparisons between layers is indicated by asterisks on the right side. B, Scatterplot of the responses shown in A. Although all layers contained RS which strongly increased firing during interactions, these were most common in L5B. In addition, L5B was nearly completely devoid of RS with strong inhibitory responses.

Table 3: Mean and median response indices of RS and FS.

| | Mean RS index | Median RS index | Mean FS index | Median FS index |
|---|---|---|---|---|
| L2/3 | 0.012±0.392 | -0.010 | 0.227±0.342 | 0.215 |
| L4 | 0.071±0.341 | 0.021 | 0.180±0.235 | 0.167 |
| L5A | -0.002±0.239 | 0.029 | 0.048±0.232 | 0.130 |
| L5B | 0.197±0.308 | 0.131 | 0.181±0.326 | 0.130 |
| L6 | 0.097±0.554 | 0.104 | 0.055±0.112 | 0.014 |

3.34) was spread between very high firing rates for L5B and L6 FS, and lower rates in units from the other layers. The overall trends of response modulation at interaction onset were similar between RS (see Fig. 3.32) and FS. Fast-spikers from L6 and L5A also modulated their activity little, while L5B showed the most consistent responses, and L2/3 and L4 increased activity, but from much lower rates. As before, to clarify the relative response change, the normalized population PSTHs for FS were also plotted (Fig. 3.36B). Figure 3.36 serves to underline the overall similarity in the touch-related modulation of RS (Fig. 3.36A) and FS (Fig. 3.36B) from the same layer.

The firing rates of FS during interactions (Fig. 3.35A) were generally comparable to those observed during the baseline period (Fig. 3.30B). The firing rates were much higher in L5B and L6 than in the other layers, and these firing rate differences were all significant. For

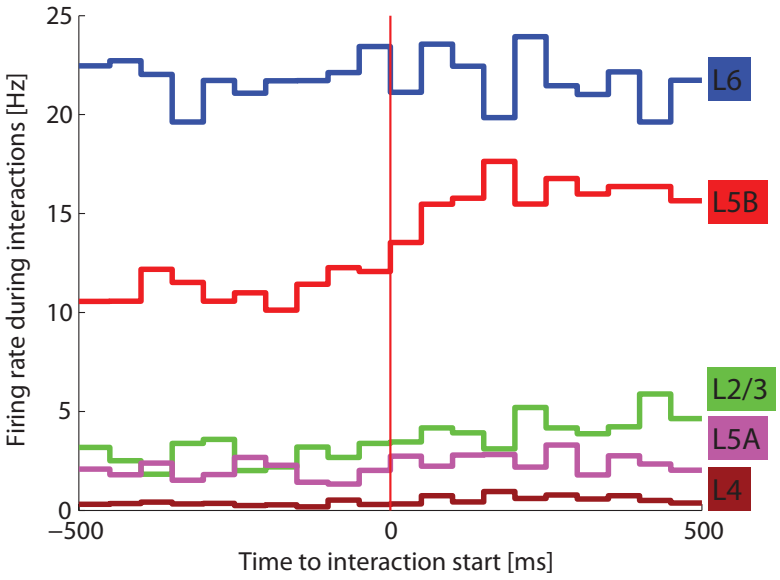



Figure 3.34: Population PSTH of responses around interaction onsets for FS, split by cortical layer.

means, standard deviations, and medians of FS firing rates during interactions see Table 2. At the level of response indices, however, there was a clear contrast between the patterns observed for RS (Fig. 3.33) and FS (Fig. 3.35B), as for FS the response indices were highest in L2/3 (mean $\approx 0.23$). Layer 4 and.L5B responses were also strongly modulated (mean $\approx$ 0.18 for both), and only L5A and L6 had relatively low mean response indices. For means, standard deviations, and medians of FS response indices, see Table 3.

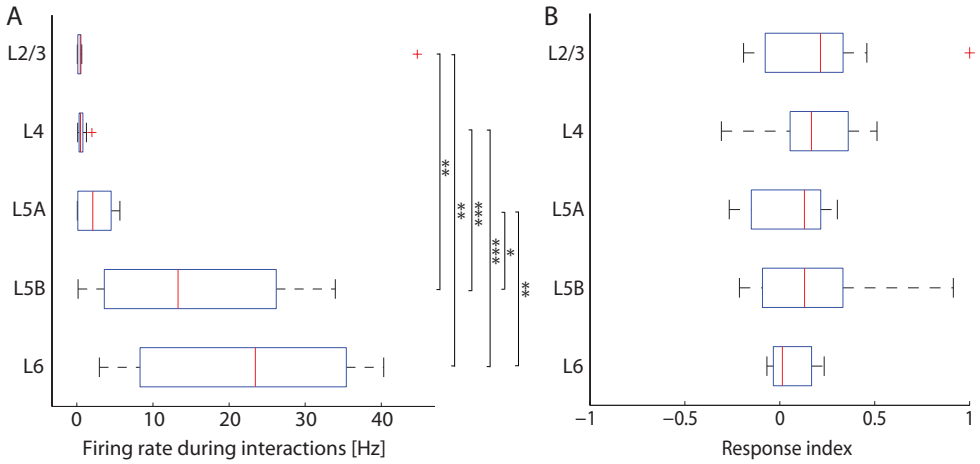



Figure 3.35: Response properties of FS during interactions as a function of cortical layer. A, In line with the baseline firing rates, evoked firing of FS was also highest in L5B and L6, and very low in L2/3 and L4. Statistical significance of evoked firing rate comparisons between layers is indicated by asterisks on the right side. B, Response indices of FS split by layer. Indices were on average higher than for RS, and were not significantly different between layers.

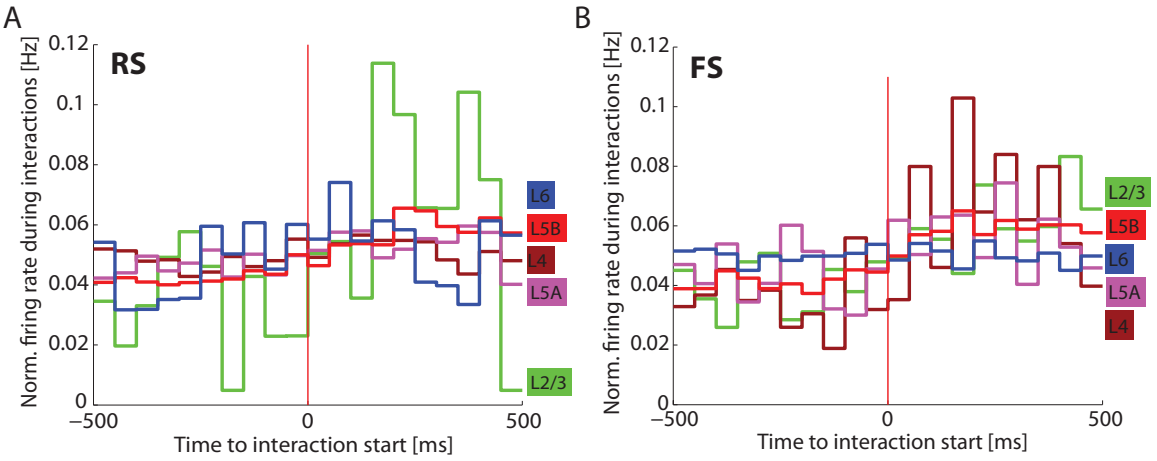



Figure 3.36: Normalized population PSTHs of responses around interaction onset. A, Population PSTH of RS responses. B, Population PSTH of FS responses.

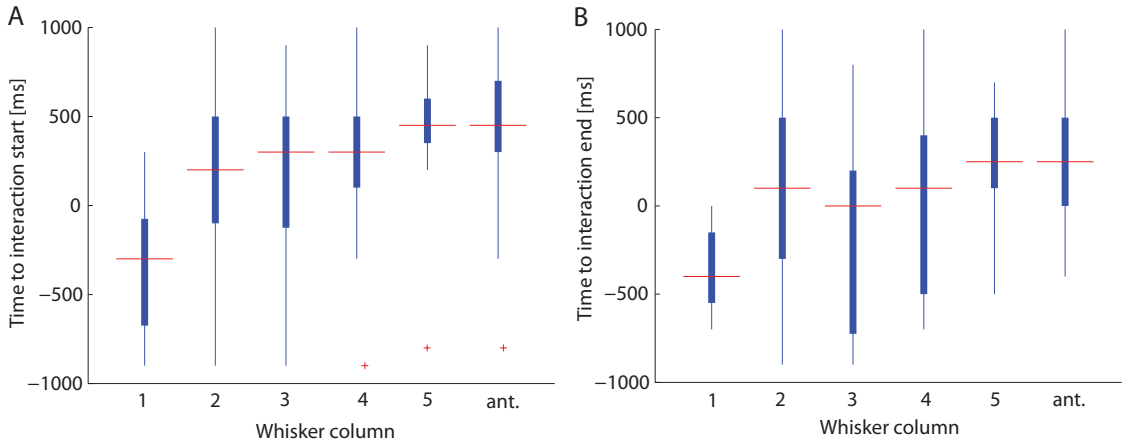



Figure 3.37: Correlation of antero-posterior RF location with the timing of neuronal responses, calculated as the first bin of a PSTH to reach the maximal value (see 2.2.2 for details). 'Whisker column' indicates the location of the RFs of corresponding units. 'ant' includes all units with RF centers anterior to column 5. A, Response timing at interaction start. Negative times indicate times before interaction start. B, Response timing at interaction end. Negative times indicate times before interaction end.

### 3.3.5 Response timing during interactions as a function of receptive fields

As units with very diverse RFs were recorded (see 3.1.1 for details), it was possible to test two complementary hypotheses: (a) neuronal responses at interaction start appear later for units with more posterior RFs, and (b) at interaction end, neuronal responses decay earlier in these units. It was surprising then that completely opposed correlations between RF location and response peak were found for the time of interaction onset and offset. For interaction onset, the opposite of the formulated hypothesis was found, in that units with posterior RFs reached their peak response earlier (Fig. 3.37A), and this effect was highly significant ($R = 0.178$, $P = 0.001$, $n = 338$, Pearson's correlation). At the same time, at interaction offset, the original hypothesis was supported by the finding that units with posterior RFs again reached their peak responses earlier within the considered time period, which is in this case equivalent to an earlier decrease in responses than in units with anterior RFs ($R = 0.129$, $P = 0.018$, $n = 331$, Pearson's correlation).

The finding that the median values of response peak times were before interaction start for column 1 whiskers (Fig. 3.37A), and after interaction end for column 2, 4, and 5, as well as anterior whiskers (Fig. 3.37B) might shed doubt on the adequacy of this analysis. However, the same analysis restricted to the time after interaction onset and before interaction offset, respectively, gave very similar results (at interaction start: $R = 0.117$, $P = 0.031$; at interaction end: $R = 0.153$, $P = 0.005$). These observations also remained largely unchanged, if bin width was increased from 100 ms to 200 ms. In this analysis, both SUs and MUs were used, but only if they fired at least 1000 spikes during the recording. See 2.2.2 for a detailed description of the units included in this analysis, how units were assigned to columns, how the response peak was found, and how the correlation was calculated.

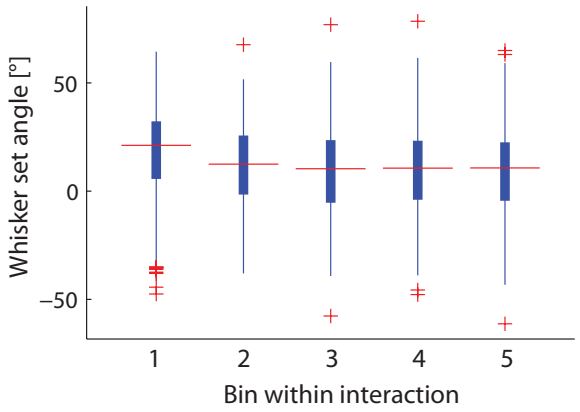



Figure 3.38: Whisker set angles decreased over the course of social interactions.

A possible explanation for the unexpected relation of response timing and RFs at the beginning of interactions might be the systematic changes in whisker set angle over the course of single interactions. At the beginning of interactions, whiskers were held more protracted than at their end (Fig. 3.38). The set angles were highly significantly different between the five sampling periods ($n = 396$ in each bin, $P < 0.0001$, Kruskal-Wallis test). The average set angle decreased from $18.4 \pm 19.8°$ during the first bin over $11.8 \pm 19.9°$ in the second to reach $9.5 \pm 19.7°$ in the third. This value did then remain stable for the remainder of the interaction. The decreases in set angle from the first to the second and from the second to the third bin were both highly significant ($P<0.001$, signed-rank test), while the set angles during the last three bins were not significantly different amongst themselves. For a description of how interaction times were binned, see 2.2.3.

### 3.3.6 Responses as a function of relative head position

As the animals were both unrestrained, interactions took place in different locations and with different relative head angles, only restricted by the widths of the two platforms and their distance. Thus, to relate neuronal responses to the relative position of stimulus to subject rat, it was necessary to transform recordings into a subject rat-centered space, as described in 2.7.3. The origin of the coordinate system in this space is the nose of the subject rat, and all rat positions as well as the positions of the rat when spikes were fired are plotted relative to it after the appropriate rotation. Figure 3.39 shows the relative head positions and spikes fired during interactions for a RS with the RF center on the D3 whisker. It can be seen that the approach occurred from different sides, and that most time during interactions was spent with the subject and stimulus rat noses in close proximity. This effect becomes even clearer in the occupancy map in Fig. 3.40A, which shows that most time was spent in nose touch. At the same time, in this particular set of interactions, there was a bias towards touch with the left side of the face.

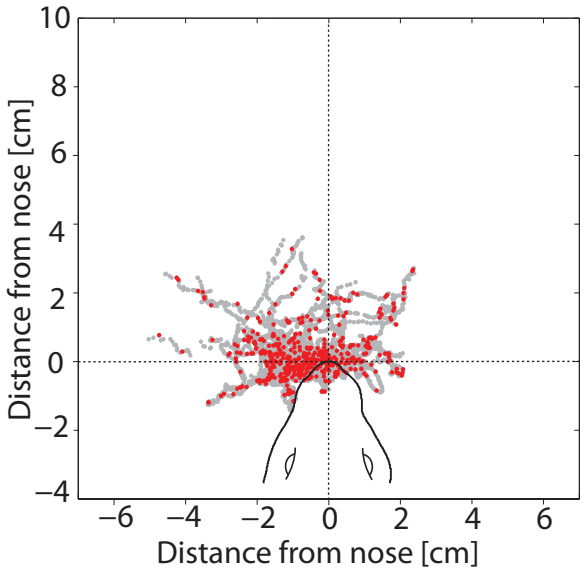



Figure 3.39: Stimulus rat nose positions during interactions (grey dots) and the spikes fired (red dots) relative to the subject rat head contour (black). Data are shown for one RS with RF on the D3 whisker.

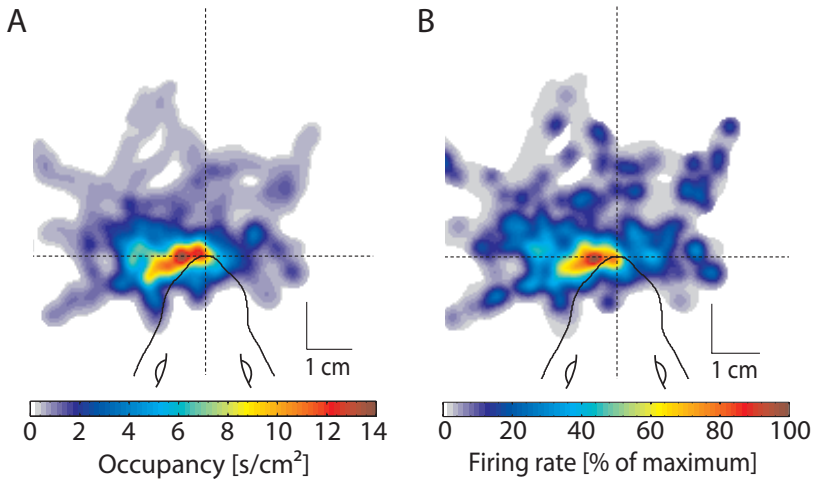



Figure 3.40: Rats spent most time during interactions in close proximity, and these were also the locations where neuronal responses were strongest. Same unit as in Fig. 3.39. A, Occupancy map, showing the summed positions of the stimulus rat nose relative to the subject rat during interactions. B, Rate map, i.e., firing normalized by occupancy time. Note the similar location of peaks in A and B.

To facilitate the interpretation of firing as a function of location, and to provide a measure of firing independent of the time spent in a certain location, normalized firing rate maps (Fig. 3.40B) were plotted. The peak in this map corresponds to the relative position of the two rats' noses to each other, where most firing of the example unit was elicited, independently of the amount of time spent in this location. It can be seen that responses were strongest when the two rats were in close proximity, with their noses touching each other.

The responses observed for a population of RS (52 units, 200 interactions), as shown in Figs. 3.41 and 3.42, were similar to those shown for an example unit in Figs. 3.39 and 3.40. The display of all sampled locations and fired spikes in Fig. 3.41 serves to show the distances from which approaches were tracked in some, although not all, interactions. It also highlights that rat heads could overlap, and in some rare cases the stimulus rat nose was up to four cm behind the subject rat nose tip. On the population level, both occupancy (Fig. 3.42A) and firing rate maps (Fig. 3.42B) were similar to the SU example above. In fact, the peak of the occupancy in Fig. 3.42A emphasizes the preference to spend time during interactions in close contact even more clearly. Similarly, the firing peak in Fig. 3.42B is spatially more confined than for the example unit, reflecting strongest activation during nose touch. There is a small but distinctive bias towards the left side to be seen in Fig. 3.42B, which is much less pronounced in the occupancy map. This presumably reflects the recording location in the right hemisphere, with the RFs being in the left whisker pad, accordingly.[3] However, this shift is weak, and overall the response fields of neurons are bilateral in Fig. 3.42B.

[3]This dataset includes one animal in which recordings were performed in the left hemisphere. In this case, the positions of occupancy and spikes fired were reflected along the head midline to assess the firing peak as a function of head position without the confounder of RFs on both sides of the face.

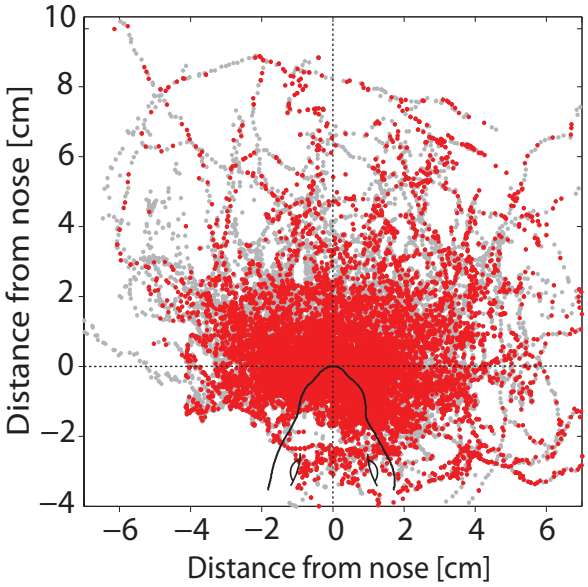



Figure 3.41: Stimulus rat nose positions during interactions (grey dots) and the spikes fired (red dots) relative to the subject rat head for 200 interactions and a population of 52 RS.

In addition, as Fig. 3.43 shows, the subject and stimulus rats not only spent most of the time in interactions with the noses close to each other, they also aligned such that the two rat midlines were parallel. Although this was partly a consequence of the platform gap, which did not allow completely lateralized approach, the occurrence of approach angles up to ca. $\pm 40°$ indicates that this was in fact a preferred alignment, and not just an artifact of the experimental setting.

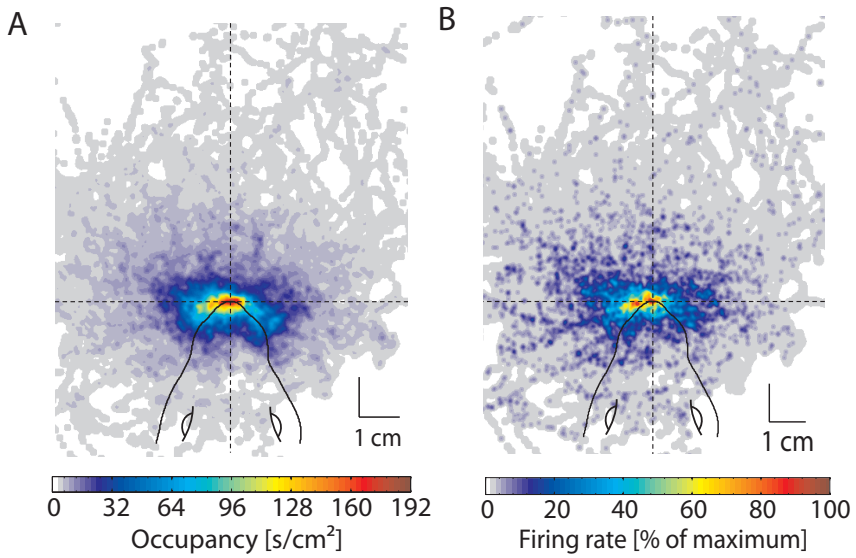



Figure 3.42: Population data for occupancy and normalized firing during interactions, recorded in both hemispheres. For recordings from the left hemisphere the image was reflected along the midline. A, Occupancy map, showing the summed positions of the stimulus rat nose relative to the subject rat during interactions. B, Rate map, i.e., firing normalized by occupancy time. Note the shift of the area of highest response to the left, i.e., contralateral to the implant.

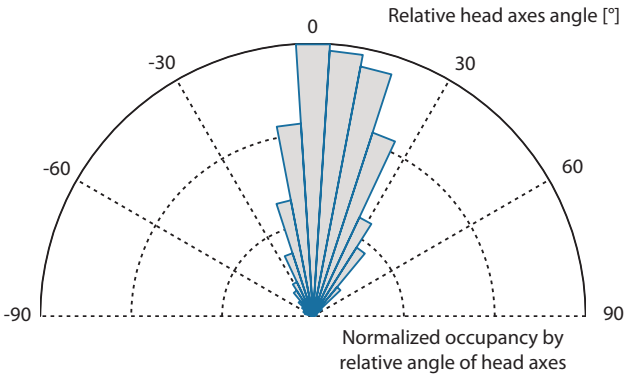



Figure 3.43: During interactions, rats were aligned with the two head midlines being parallel. An angle of 0° corresponds to no angle between the two head midlines (see Fig. 2.5 for details).

### 3.3.7 Subject trimming

To investigate whether the observed responses in BC are of tactile origin, trimming experiments were performed (see 2.1.3; schematic in Fig. 3.44).

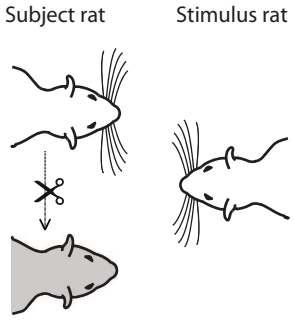



Figure 3.44: Schematic of subject trimming experiments.

As explained in the methods, trimming comparisons were either 'within-cells' (paired) or 'between-cells' (unpaired). In the former, recordings from the same day before and after trimming are compared, in the latter, all units where the rat was trimmed or untrimmed, respectively, are compared. Both comparisons showed the same outcome, a decrease of response modulation after trimming. This can be seen for an example MU in Fig. 3.45, which increased firing during interactions as long as the rat was untrimmed, but stopped doing so after trimming.

On the population level for the within-cells comparison, firing rates during interactions decreased consistently after whisker trimming (Fig. 3.46A). This effect was seen in 11 out of 12 units, which was a significant difference ($P = 0.007$, signed-rank test). The only unit which did not show this effect was a FS which fired at a rate of ca. 40 Hz and minimally increased activity (not shown in Fig. 3.46A). For this comparison, SU and MU were combined to obtain a sufficient number of units. The necessity to do so arose from the triple complication of (i) trimming an awake animal, (ii) obtaining a sufficient number of interactions after trimming, and (iii) getting recordings with good signal-to-noise ratio towards the end of an experiment. Thus, the number of SU recorded was low. The last two complications also contributed to a low number of units in the between-cells comparison. Here, the disappearance of responses at interaction onset was equally clear for all three unit types (RS in Fig. 3.46B, FS in Fig. 3.46C, and MU in Fig. 3.46D). Thus, the mean response ratio (firing during interactions divided by baseline) of all units decreased from $1.35 \pm 1.09$ for untrimmed animals ($n = 601$)

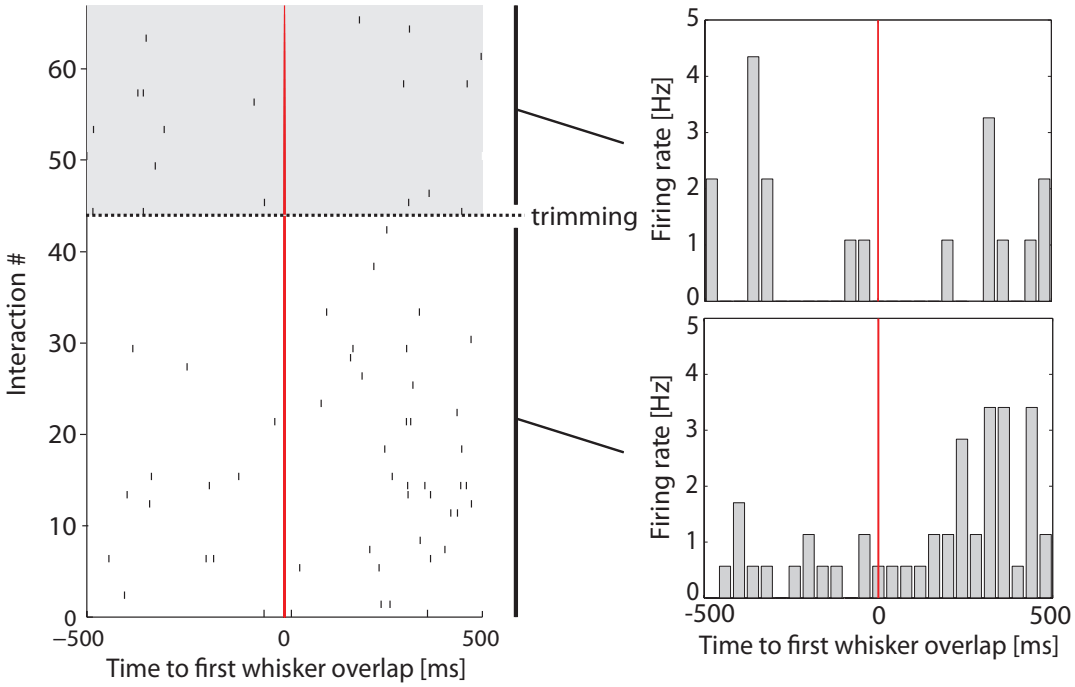



Figure 3.45: The response of an example MU decreases after whisker trimming. The response increase seen in the PSTH before trimming (lower right) disappears after trimming (top right), which can also be seen in the raster plot on the left side. The gray shaded area corresponds to the interactions which took place after trimming.

to 0.99±0.25 (n = 17) after trimming (P = 0.161, U test).[4] In contrast to the within-cells analysis, in the between-cells analysis the absolute firing rates are not directly comparable, which is aggravated by the low number of cells recorded after trimming. Thus, the five FS recorded after trimming had an average firing rate much above those recorded before trimming (Fig. 3.46C).

### 3.3.8 Stimulus trimming

Complementary to the subject trimming experiments, neuronal signals during touch of trimmed and untrimmed stimulus animals were also compared to investigate further the origin of interaction-related firing in BC (schematic in Fig. 3.47).

[4] In this trimming dataset, one outlier with only 232 spikes overall and a ratio of 30.2 has been excluded. The discrepancies between the number of units in this analysis and the ones shown in Fig. 3.46 are explained by the usage of the more liberal stability threshold when MUs were included. It should also be noted that in the between-cells analysis, those unit responses from trimmed rats, which were already used in the within-cells analysis, were not included.

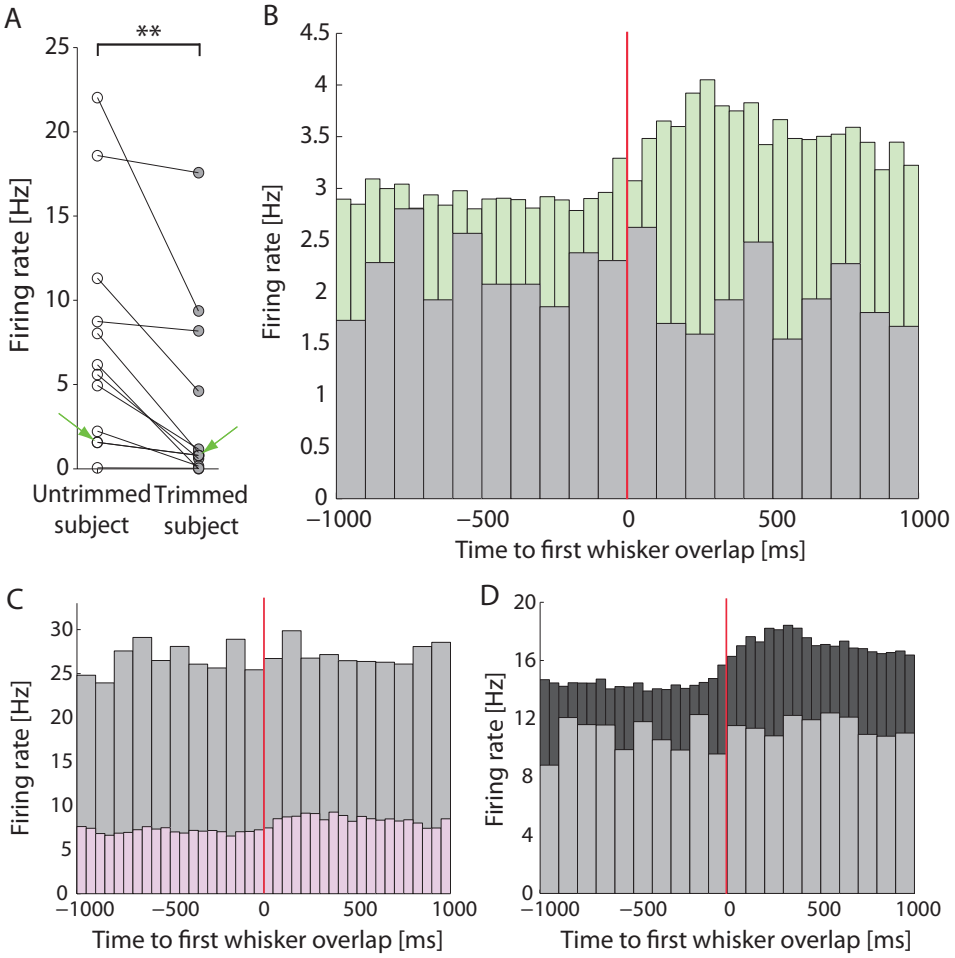



Figure 3.46: Whisker trimming abolishes responses. A, Within-cell comparison between firing rates in interactions before and after trimming. The responses decreased for all units, except a FS which fired with ca. 40 Hz and is not included in the plot. The example unit shown in Fig. 3.45 is marked by green arrows. B, Population PSTHs of RS before trimming (green, n = 242) and after trimming (light gray, n = 6). Firing rates decreased and the response increase at interaction onset is absent after trimming. Binning has been adjusted to the small number of units from trimmed rats. C, Same as B for FS before (violet, n = 72) and after trimming (light gray, n = 5). D, Same as B for MU before (dark grey, n = 247) and after trimming (light gray, n = 6). The increase at interaction onset is visible before trimming for both FS (C) and and MU (D). After trimming this increase disappears. Note that the FS recorded from trimmed animals fired at much higher baseline rates than the much larger FS population recorded from untrimmed animals.

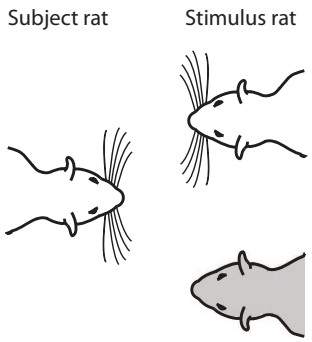



Figure 3.47: Schematic of stimulus trimming experiments.

Surprisingly, there was relatively little influence of the stimulus trimming state onto firing rates of the subject rat's BC neurons. This can be seen for the example neuron in Fig. 3.48A, as well as for the population of recorded SUs in Fig. 3.48B. Mean firing rates of RS were very similar during touch of untrimmed rats (1.45±3.10 Hz), and trimmed rats (1.36±3.03 Hz), and medians were nearly identical (0.27 Hz for untrimmed, 0.28 Hz for trimmed; P = 0.600, n = 41, signed-rank test). However, the bias of FS towards higher firing rates during interactions with untrimmed rats was also weak, but much more consistent (means 6.20±9.77 vs. 5.51±9.27 Hz, medians 1.97 vs. 1.77 Hz; P = 0.023, n = 20, signed-rank test). As a consequence, the pooled SU population also had a bias towards stronger firing during touch of untrimmed animals, but this was not significant (P = 0.060, n = 61, signed-rank test).

As can be seen in Fig. 3.48A, some units which did not differ in overall firing rates as a function of stimulus trimming state, nevertheless indicated in their response timing that stimulus triming had an influence on how precisely BC neurons responded during interactions. For a discussion of how these effects could come about, see 4.3.9.

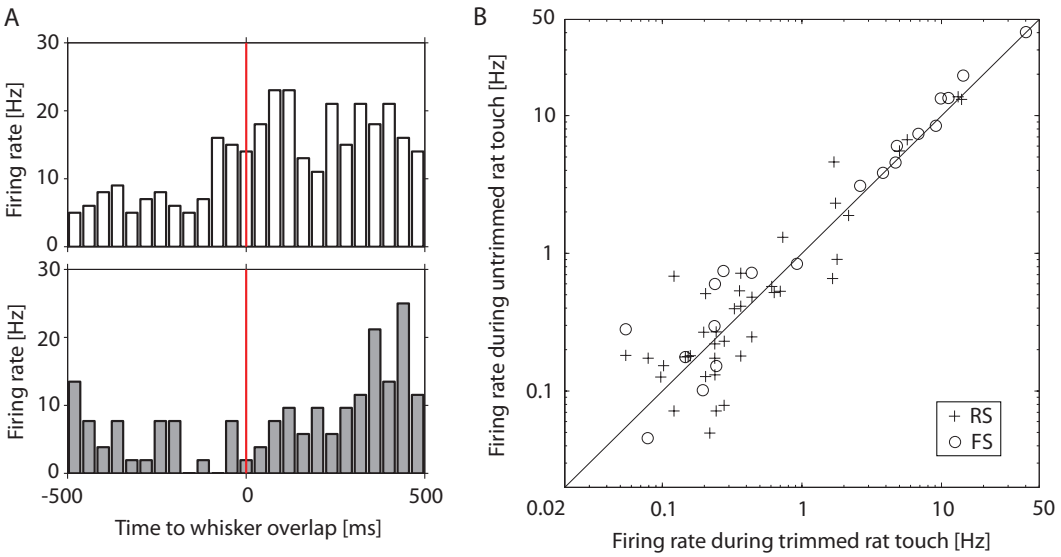



Figure 3.48: Comparison of responses during touch of trimmed and untrimmed stimulus animals. A, An example RS shows a strong response increase during touch of both untrimmed (top) and trimmed stimulus rats (bottom). However, it is also noticeable that the response to the touch of trimmed stimulus animals begins later than to untrimmed stimuli. B, On the population level, neuronal touch responses were only weakly modulated by the trimming state of stimulus animals.

### 3.3.9 Object touch responses

To investigate whether responses to social touch are different from other touch events, and if so, how, object touch responses were compared with responses during social interactions (schematic in Fig. 3.49).

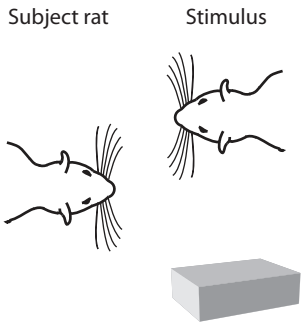



Figure 3.49: Schematic of the experiments with object and alive rat presentation. Objects and alive rats were not presented in a chooser setting, but rather sequentially.

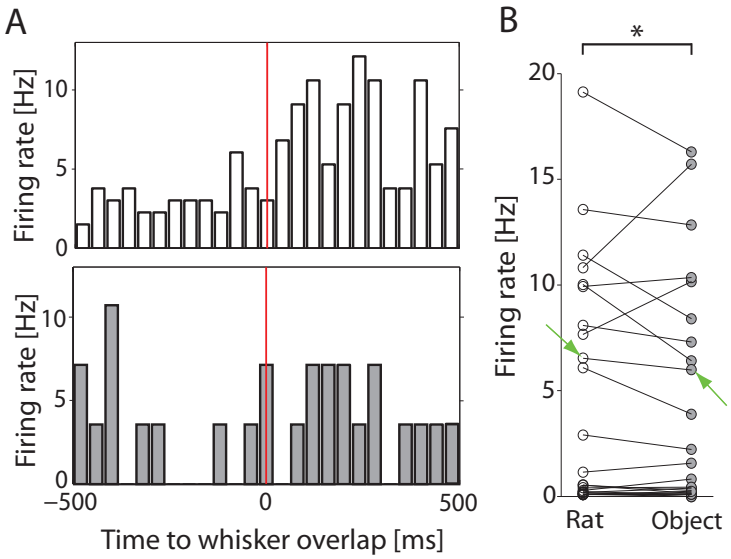



Figure 3.50: Comparison of responses during touch of alive rats and objects. A, PSTH of the response of a RS to social touch (top) and object touch (bottom). B, Firing rates during social touch are higher than during object touch. The plot shows a randomly drawn subset of one third of the data, while statistics is reported for the whole RS population. The example unit shown in A is highlighted by green arrows.

Rats showed less interest in objects[5], but even when they touched them, responses were weaker than during alive rat touch. Figure 3.50A compares the responses of an example RS during touch of these two stimuli, and shows that responses during alive rat touch were much stronger at interaction onset. This effect was also observed consistently on the population level. Median firing rates of RS were 1.33 Hz during alive and 0.83 Hz during object interactions ($P = 0.023$, $n = 92$, signed-rank test; see Figs. 3.50B and 3.51). The corresponding means were 4.04±5.15 Hz and 3.59±4.64 Hz for alive and object touch, respectively.

These response differences were observed not only for RS, but also for FS (Fig. 3.51). Thus, the mean firing rate of FS during alive rat touch was 2.51±3.38 Hz, and 2.13±3.10 Hz during object touch (medians 0.69 Hz vs. 0.45 Hz; $P = 0.123$, $n = 22$, signed-rank test). As a consequence, when pooling all units, the significance level of the comparison between alive rat and object responses increased further ($P = 0.007$, $n = 114$, signed-rank test). It should be noted that within the set of units recorded while objects were presented, FS had in fact a lower average firing rate than RS.

[5]It should be noted that this qualitative behavioral observation was not supported by the quantification of interaction times, as presented in 3.2.4. For reasons why a preference to interact with alive rats over objects might not be reflected in interaction times, see there.

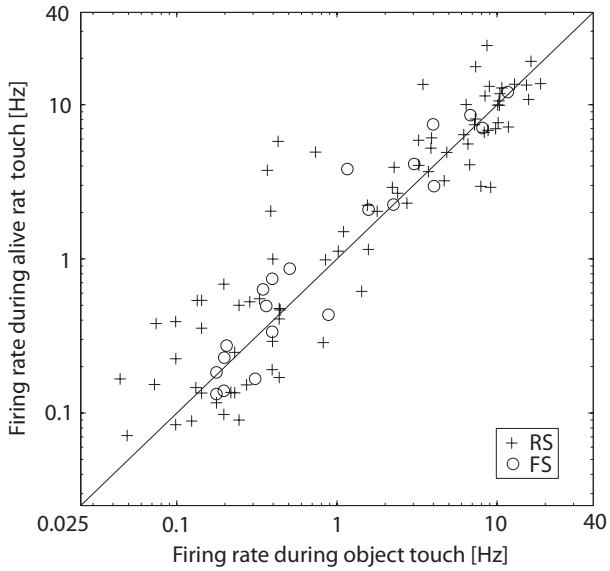



Figure 3.51: The responses during alive rat touch are weakly but consistently higher than during object touch for both RS and FS.

### 3.3.10 Stuffed rat touch responses

As a further comparison to the touch of alive rats, a stuffed rat (see 2.1.4) was presented (schematic in Fig. 3.52). For behavioral differences between alive and stuffed rat touch, see 3.2.3.

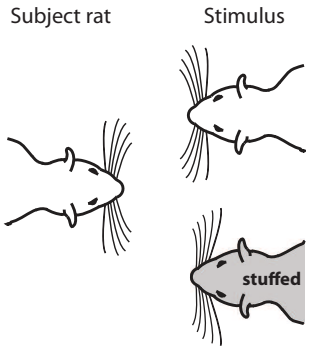



Figure 3.52: Schematic of the experiments with stuffed and alive rat presentation. Stuffed and alive rats were not presented in a chooser setting, but rather sequentially.

Although interactions with stuffed and alive rats were very different regarding both whisking parameters and measures of interest, on the level of physiological responses the differences

between stuffed and alive rat touch were weak (Fig. 3.53). Although some units showed much stronger responses during alive than stuffed rat touch (Fig. 3.53A), the average firing rates of RS during alive rat touch (2.38±3.46 Hz) and stuffed rat touch (2.31±3.71 Hz) were very similar (Fig. 3.53B). However, the bias towards higher alive rat responses was stronger for the medians (0.52 Hz vs 0.38 Hz), and this difference was significant (P = 0.048, n = 87, signed-rank test). The 20 FS, for which interactions with both alive and stuffed rats were observed, did not fire differentially, and when all SUs were combined, the effect observed for RS only was not significant (medians 0.54 Hz vs. 0.41 Hz, P = 0.069, n = 107, signed-rank test). Overall, responses to the stuffed rat seem to be slightly weaker than those to alive rats, but less so than object responses. See 4.3.10 for a discussion of the effects described here and in 3.3.9.

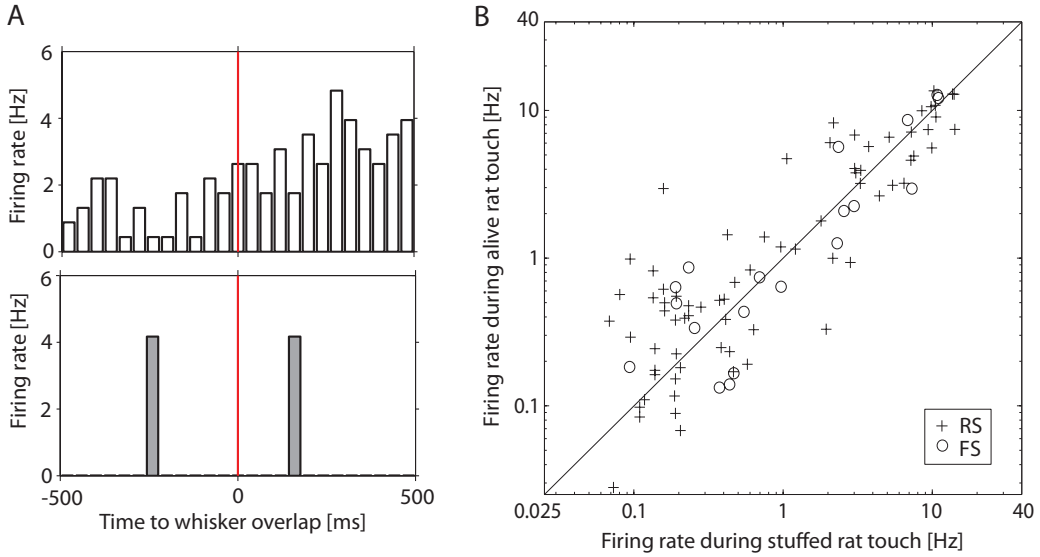



Figure 3.53: Comparison of responses during alive and stuffed rat touch. A, An example RS shows a strong response increase during alive rat touch (top), but no response to stuffed rat touch (bottom), as well as a generally lower firing rate around interactions with the stuffed rat. B, On the population level, the responses during alive rat touch were only weakly higher than during stuffed rat touch.

### 3.3.11 Anesthetized rat touch responses

The difference between the touch of alive and stuffed rats was only minimal, and whisker trimming of the stimulus animals had no visible effect. To further investigate, which stimulus parameters were important in eliciting a response by BC neurons, an anesthetized rat (see 2.1.5) was additionally presented in a subset of experiments (schematic in Fig. 3.54).

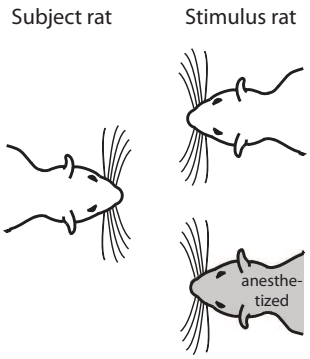



Figure 3.54: Schematic of the experiments with anesthetized and alive rat presentation. Anesthetized and alive rats were not presented in a chooser setting, but rather sequentially.

The responses during touch of an anesthetized rat were remarkably similar to those elicited by the touch of awake, behaving rats, i.e., social interactions. Both the RS and the FS responses were very similar for these two types of stimuli (Fig. 3.55). Average RS responses were 1.43±3.49 Hz (median 0.29 Hz) for awake and 1.34±3.46 Hz (median 0.26 Hz) for anesthetized rats. Not only were the medians very similar, but the two responses were extremely good predictors of each other ($R = 0.988$, $P < 0.0001$, Pearson's correlation). Accordingly, the firing rate differences were not significant ($P = 0.987$, $n = 15$, signed-rank test).

The picture for FS was very similar, with mean firing rates of 2.86±4.72 Hz (median 0.43 Hz) for awake and 2.89±5.08 Hz (median 0.32 Hz) for anesthetized stimulus rats. Again, the correlation of responses was very strong ($R = 0.994$, $P < 0.0001$, Pearson's correlation) and the response difference was not significant ($P = 1.000$, $n = 8$, signed-rank test).

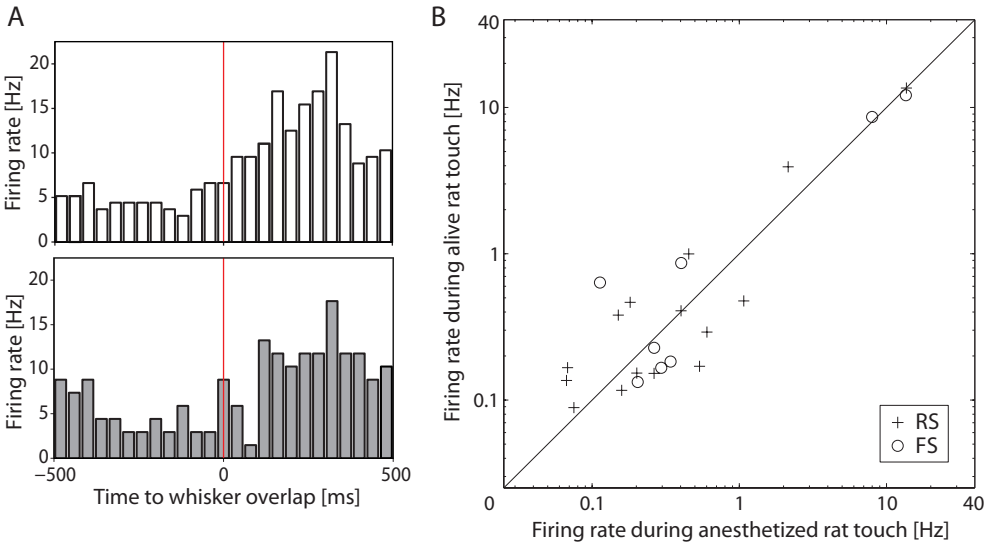



Figure 3.55: Comparison of responses during anesthetized and alive rat touch. A, An example FS shows a similarly strong response increase during alive (top) and anesthetized rat touch (bottom). B, On the population level, the responses during alive and anesthetized rat touch were very similar.

### 3.3.12 Sex-specificity of interaction-related responses

Because FS were much fewer in number, the analysis of the sex-specificity of responses has been restricted to RS. In this analysis, different stimulus animals have been considered individually, such that for one recorded neuron, responses were considered separately for all stimulus partners which the rat interacted with on that day. A prerequisite for inclusion was that at least three interactions with the respective animal took place. Throughout the figures in this paragraph, blue and red indicates male and female stimulus animals, respectively. The terms 'male neuron' and 'female neuron' are used to refer to neurons recorded in male and female rats, respectively. 'Non-estrus' includes all phases of the hormonal cycle except for the estrus phase.

**Firing rates** Responses to social touch observed in male neurons were often strong and similar for the touch of male and female stimulus rats, as shown for an example neuron in Fig. 3.56A,B. In contrast, female neurons showed overall weaker modulations, which were, in addition, often different for male and female stimulus rats (Fig. 3.56C,D).

On the population level, male RS showed strong response modulations, in particular strong excitatory responses (Fig. 3.57). The average baseline firing rate of male RS of 2.33±3.41 Hz (median 0.93 Hz) increased to 3.35±4.85 Hz (median 1.13 Hz) during social interactions. This corresponded to an overall 44% increase in responses. The median firing rates increased from 0.93 Hz to 1.13 Hz, a 22% increase. The interaction firing rates were similar for the touch of male (mean 3.38±5.28 Hz, median 1.14 Hz) and female stimulus animals (mean 3.29±4.97 Hz, median 0.77 Hz), and this difference was not significant ($P = 0.396$, $n = 152$ for males, $n = 143$ for females, U test). Baseline firing rates during the presentation of male and female stimulus rats were also similar (means 2.31±3.58 Hz for males and 2.06±3.25 Hz for females; $P = 0.424$, U test). The presence of differences in baselines is explained by the periods considered for baseline calculations. These always included only those presentation periods where the individual stimulus rat in question was presented.

Female RS responses were overall much weaker than responses of male RS. This was seen in females regardless of whether they were in non-estrus (Fig. 3.58A) or in estrus (Fig. 3.58B). Thus, the average response of female RS independently of estrus state increased from 3.14±4.00 Hz to 3.73±4.76 Hz during interactions, an increase by 19%. The medians of firing rates increased by 7% from a baseline rate of 1.66 Hz to an evoked firing rate of 1.77 Hz. No significant differences were observed in the firing rates of female cells between interactions with male and female stimulus rats. However, it should be noted that median interaction firing rates of female cells were much higher when they interacted with males (2.09 Hz) than

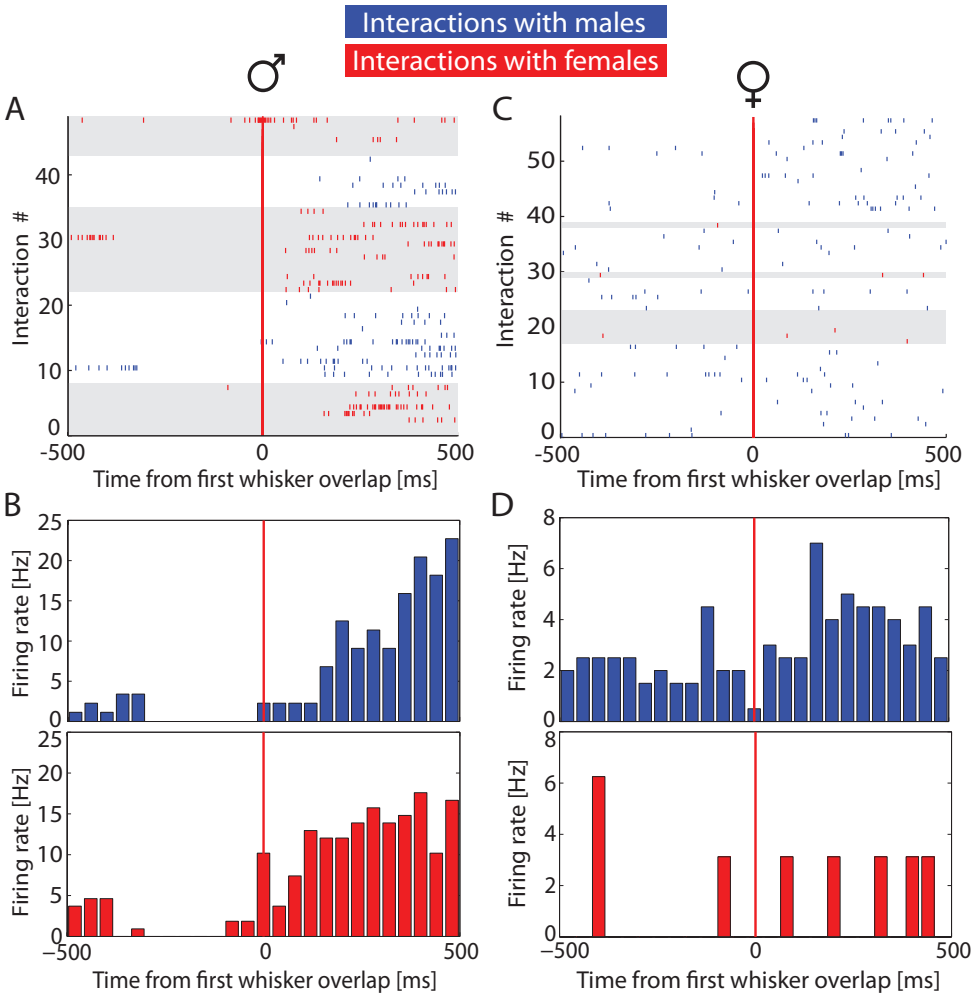



Figure 3.56: Responses during interactions with males are depicted in blue, and with females in red. A, Raster plot of the responses of a RS recorded from male BC around interaction onset. B, PSTHs of the responses shown in A for the interactions with males (top) and females (bottom). Note the strong and similar responses in interactions with both sexes. C,D, Similar to A,B for a RS from female BC. The unit shows weak modulation during interactions, as well as a generally decreased firing rate around interactions with females. Also note the strong behavioral preference to interact with males.

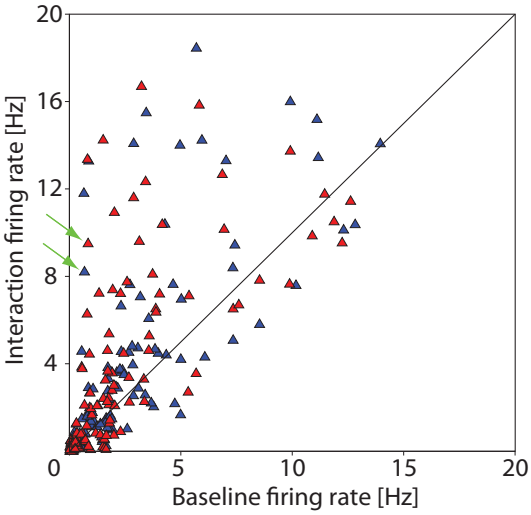



Figure 3.57: Scatterplot of RS responses in male BC during facial interactions against baseline (outside of interactions). Note the presence of units with strong response increases during interactions. The example unit shown in Fig. 3.56A,B is highlighted by green arrows for two stimulus animals.

with females (0.88 Hz), and this comparison nearly reached significance (P = 0.053, n = 190 for males, n = 176 for females, U test).

Furthermore, it was observed that the estrus state of the female was reflected in the firing rates of neurons. Both mean baseline firing (3.46±4.41 Hz in non-estrus vs 1.77±2.52 Hz in estrus) and mean interaction firing rates (3.76±4.71 Hz in non-estrus vs 2.24±3.37 Hz in estrus) varied with estrus cycle, and were much higher when the female was in non-estrus. The corresponding median values were also much higher in non-estrus than in estrus for both baseline (2.06 Hz vs 0.59 Hz) and interaction firing rates (1.98 Hz vs 0.47 Hz). In this analysis, each cell was considered as one entry, and the reported differences were not significant ($P > 0.1$ for interaction and baseline firing rates, n = 32 for estrus and n = 60 for non-estrus, U test). When, in contrast, each stimulus animal was considered individually, the interaction firing rate in non-estrus was significantly higher than in estrus (P = 0.002, n = 193 for non-estrus and n = 92 for estrus, U test). The corresponding mean interaction firing rates considering individual stimulus animals were 3.93±4.99 Hz (median 1.85 Hz) in non-estrus and 2.29±3.62 Hz (median 0.39 Hz) in estrus.

None of the firing rate differences between responses to male and female stimulus animals was significant, whether for estrus or non-estrus females, and whether based on individual cells or individual stimulus animals.

Table 4 details the means and medians for both interaction and baseline firing rates, split by the sex of the subject animal, as well as the estrus state of females, and both split and not split by stimulus animal sex.

Table 4: Mean and median firing rates of RS during and outside of interactions, split by subject sex, as well as both split and unsplit by stimulus sex. Note that for the responses not split by stimulus rat sex ('alive rat'), responses to all stimulus rats from a given day have been pooled, and there are as many entries as cells. For the analysis of the effect of stimulus partner sex on responses, however, each stimulus partner which fulfilled the interaction criterion is considered independently. In addition, the % change from baseline to evoked firing rate, and the number of entries are given. F = female, M = male, ES = estrus, NO = non-estrus; first letter is the subject, second the stimulus rat.

| | Mean interaction rate [Hz] | Mean baseline rate [Hz] | % change | Median interaction rate [Hz] | Median baseline rate [Hz] | % change | n |
|---|---|---|---|---|---|---|---|
| M_alive | 3.35±4.85 | 2.33±3.41 | 44 | 1.13 | 0.93 | +22 | 108 |
| M_M | 3.38±5.28 | 2.31±3.58 | 46 | 1.14 | 0.94 | 21 | 152 |
| M_F | 3.29±4.97 | 2.06±3.25 | 60 | 0.77 | 0.84 | -8 | 143 |
| F_alive | 3.73±4.76 | 3.14±4.00 | 19 | 1.77 | 1.66 | +7 | 134 |
| F_M | 4.01±4.83 | 3.31±3.95 | 21 | 2.09 | 1.70 | +23 | 190 |
| F_F | 3.41±4.52 | 2.97±3.89 | 15 | 0.88 | 1.06 | -17 | 176 |
| F_alive_NO | 3.76±4.71 | 3.46±4.41 | 9 | 1.98 | 2.06 | -4 | 60 |
| F_M_NO | 4.44±5.21 | 3.77±4.40 | 18 | 2.36 | 2.22 | 6 | 96 |
| F_F_NO | 3.43±4.73 | 3.02±4.10 | 14 | 0.96 | 1.06 | -9 | 97 |
| F_alive_ES | 2.24±3.37 | 1.77±2.52 | 27 | 0.47 | 0.59 | -20 | 32 |
| F_M_ES | 2.30±3.54 | 1.94±2.91 | 19 | 0.49 | 0.38 | 29 | 40 |
| F_F_ES | 2.28±3.75 | 1.86±3.00 | 23 | 0.35 | 0.29 | 21 | 42 |

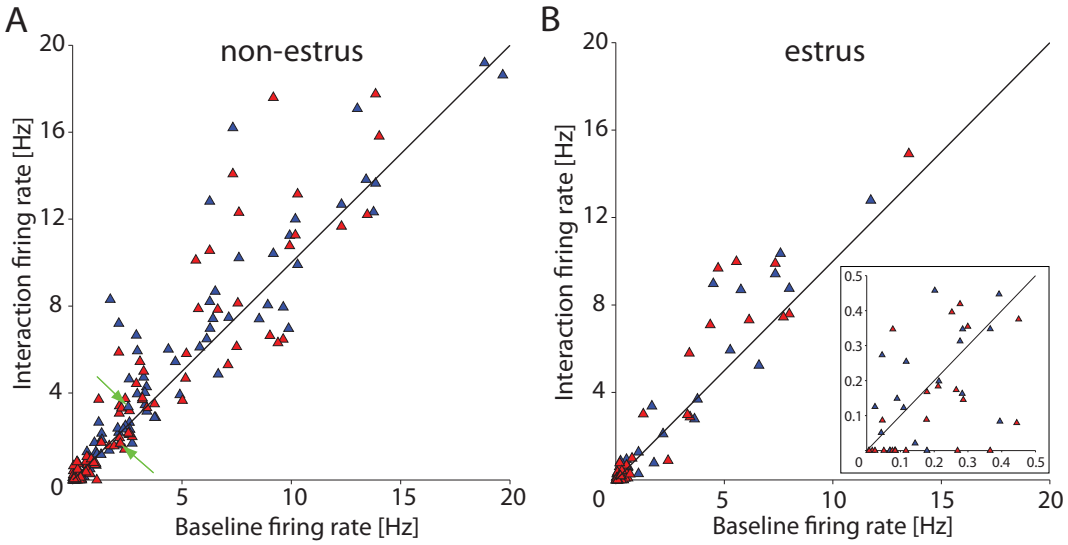



Figure 3.58: A, Scatterplot of RS responses in female BC during facial interactions against baseline, for subject females in non-estrus. The example unit shown in Fig. 3.56C,D is highlighted by green arrows for two stimulus animals. B, Same as A for females in estrus. Note the lower firing rates, as well as the inhibition exhibited by low-firing cells during interactions (inset).

Table 5: Mean and median values of reponse indices, split by subject rat sex, as well as the estrus state for subject females. The value for the probability that the median of the index distribution was not different from zero (one-sided signed-rank test) is also given, as well as the number of entries. F = female, M = male, ES = estrus, NO = non-estrus; first letter is the subject, second the stimulus rat.

| | Mean index | Median index | n | P-value vs. 0 |
|---|---|---|---|---|
| M_M | 0.241±0.459 | 0.253 | 152 | <0.0001 |
| M_F | 0.174±0.521 | 0.172 | 143 | <0.0001 |
| F_M_ES | 0.076±0.472 | 0.060 | 40 | 0.213 |
| F_F_ES | -0.252±0.541 | -0.085 | 42 | 0.013 |
| F_M_NO | 0.072±0.425 | 0.002 | 96 | 0.168 |
| F_F_NO | 0.083±0.462 | 0.032 | 97 | 0.090 |

**Indices** Firing rates do not reveal the stimulus selectivity very well, and thus response indices were calculated to capture stimulus selectivity independently of firing rates. As visible in Fig. 3.59, the stronger modulations of male RS were reflected in higher average indices. Average indices of male RS were 0.241±0.459 for male and 0.174±0.521 for female stimulus animals. In contrast, mean female indices never exceeded 0.1. These much higher indices in male RS occur without a difference in firing rate (see Table 4). The difference between male (3.35±4.85 Hz) and female evoked rates (3.73±4.76 Hz) was not significant (P =0.117, U test).

Importantly, the female RS response indices, when split by both stimulus rat sex and estrus state of the subject rat, indicated an interaction between both factors. Only for interactions of females in estrus with other females was an inhibition of neuronal responses observed (mean index = -0.252±0.541). In contrast, interactions with males elicited responses which were weakly excitatory (mean index = 0.076±0.472), and thus similar to responses of non-estrus females to both stimulus males (0.072±0.425) and females (0.083±0.462). However, it should also be noted that the inhibitory effect, seen for non-estrus female interactions with other females only, is predominantly due to very low-firing cells, some of which did not fire any spikes at all during interactions with females (Fig. 3.58B, inset), and thus produced index values of -1. The mean and median firing rates listed in Table 4 also do not clearly reflect the inhibitory effect found for indices.

A summary of these response patterns, which were partly associated with highly significant differences between groups, is shown in Fig. 3.59. Table 5 and Table 6 report the index population data and the P-values of comparisons, respectively.

Table 6: Cross-table of significance levels for the comparisons between response indices of RS from females in estrus, females in non-estrus, and males. Significant comparisons are marked by asterisks in Fig. 3.59. F = female, M = male, ES = estrus, NO = non-estrus; first letter is the subject, second the stimulus rat.

| | F_M_ES | F_F_ES | F_M_NO | F_F_NO | M_M | M_F |
|---|---|---|---|---|---|---|
| F_M_ES | x | 0.010 | 0.623 | 0.977 | 0.049 | 0.230 |
| F_F_ES | 0.010 | x | 0.005 | 0.002 | <0.001 | <0.001 |
| F_M_NO | 0.623 | 0.005 | x | 0.635 | 0.002 | 0.031 |
| F_F_NO | 0.977 | 0.002 | 0.635 | x | 0.005 | 0.101 |
| M_M | 0.049 | <0.001 | 0.002 | 0.005 | x | 0.298 |
| M_F | 0.230 | <0.001 | 0.031 | 0.101 | 0.298 | x |

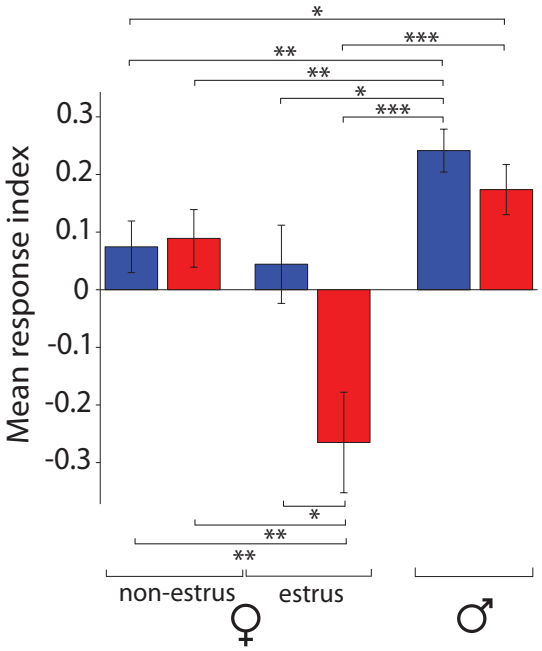



Figure 3.59: Population data for response indices of BC RS (+1 = maximal excitation, -1 = maximal inhibition, 0 = no change of activity in interactions). Response indices of RS recorded in female rats were generally lower than those recorded from males, and were on average negative when females in estrus interacted with other females, marking inhibition. Error bars indicate standard errors of the mean.

To investigate whether the unequal distribution of recorded cells from males and females over different layers could be the cause of the observed sex-specific differences in response modulations, responses from L5B RS alone were compared. As these were much more numerous in the subset of male RS with identified layer (Fig. 3.6) and had the highest response indices (Fig. 3.33), this could be a confounder in the interpretation of these results (see 4.3.11). This comparison indicates, however, that the higher response indices of male RS were properties of male RS independent of layer. Thus, the mean response ratio (interaction firing rate divided by baseline firing rate) of male RS was 2.13±2.37 (median = 1.39), much higher than for females (mean = 1.26±0.38, median = 1.15). The number of female L5B RS was small, however, and the difference was not significant (n = 56 for males, n = 9 for females; P = 0.575, U test). When response indices were calculated for each stimulus rat, as was the case for other analyses in this section, the response indices were also much higher for male than female L5B RS. Male RS had a mean response index of 0.293±0.515 (median 0.253), while the female RS mean index was only 0.097±0.242 (median 0.069), and the difference was significant in this case (n = 176 for male cells, n = 29 for female cells; P = 0.021, U test).

### 3.3.13 Relation of whisking behavior and neuronal responses

To address the question whether the sex-specific differences in responses described in 3.3.12 could be explained by differential whisking behavior, whisking parameters of both subject and stimulus rat and neuronal response strength were correlated for individual interactions. As parameters of whisking behavior, the whisker set angle during interactions and the whisking power in the 8-12 Hz range were chosen. Whisking amplitude was also quantified initially, but as it was very strongly correlated with whisking power, it was not considered further. Response strengths were quantified as response indices. These differ from the indices reported in previous sections throughout this work, in that the baseline time was the complete time of recordings except for interaction times. However, when the indices were calculated based on matched cutouts of the baseline time, similar to the indices used in other analyses, the outcome was not very different from the one presented here, except for a high number of undefined indices, as well as indices with values of +1, in particular for the example unit shown in Figs. 3.60A,B and 3.61A,B. For a detailed explanation of the two alternative index calculations, see 2.7.2.

The example unit shown in Fig. 3.56A,B, which was recorded from a male rat, showed a strong response increase during interactions and only sparse firing outside of interactions. As to be expected, when response indices of this unit were calculated for single unteractions, these were typically very high and close to +1 for many interactions (Fig. 3.60A). Indices were high regardless of whisking power during the specific interaction, which varied by over threefold. In fact, the four interactions with lowest indices corresponded to interactions with both very high and very low whisking power. Similarly, there was no strong correlation between the whisker set angle and response index for this unit (Fig. 3.60B). The example unit recorded from a female rat, shown in Fig. 3.56C,D, exhibited much weaker modulation during interactions,

and, as a consequence, indices were also much lower. Although the spread here was higher than for the male neuron, the correlations between indices and whisking parameters were weak for both whisking power (Fig. 3.60C) and whisker set angle (Fig. 3.60D). In this and the next figures (Figs. 3.60-3.63), the strength of correlation between whisking measures and indices, and its significance level, are given within the respective figure panel.

When the whisking power or set angle of the stimulus rat were correlated with neuronal responses of the subject rat, the same picture emerged for both the example male neuron (Fig. 3.61A,B) and the example female neuron (Fig. 3.61C,D). The whisking behavior of stimulus rats was not a strong predictor of the responses of these particular units.

The sample of interactions for each example unit was relatively small, and as some correlations reached an absolute correlation value of ca. 0.3 (and, correspondingly, an $R^2$ of ca. 0.1), it was conceivable that an effect was missed due to low statistical power. Thus, the same correlation was performed for the whole dataset of 200 tracked social interactions. When response indices were correlated with whisking measures for each RS present on the respective day of recording, the dataset contained 865 combinations of unit and interaction. In this dataset, correlations were presumably much more representative.

Overall, correlations were always weak on the population level as well, both for measures of subject rat whisking (Fig. 3.62) and stimulus rat whisking (Fig. 3.63). Absolute correlation values never exceeded 0.14, and were over 0.1 for only one comparison. Thus, even for the strongest correlation, which was between whisking power of subject males and response indices (Fig. 3.62A), under 2% of the variation in the indices was explained by a whisking parameter. This correlation was the only one significant ($P = 0.004$) amongst eight comparisons. At the same time, the fact that this correlation remained significant after correction for multiple comparisons ($P = 0.032$, Bonferroni-corrected) suggests that responses in male BC during social interactions might be weaker when whisking power is high. For female cells such a negative correlation was nearly absent.

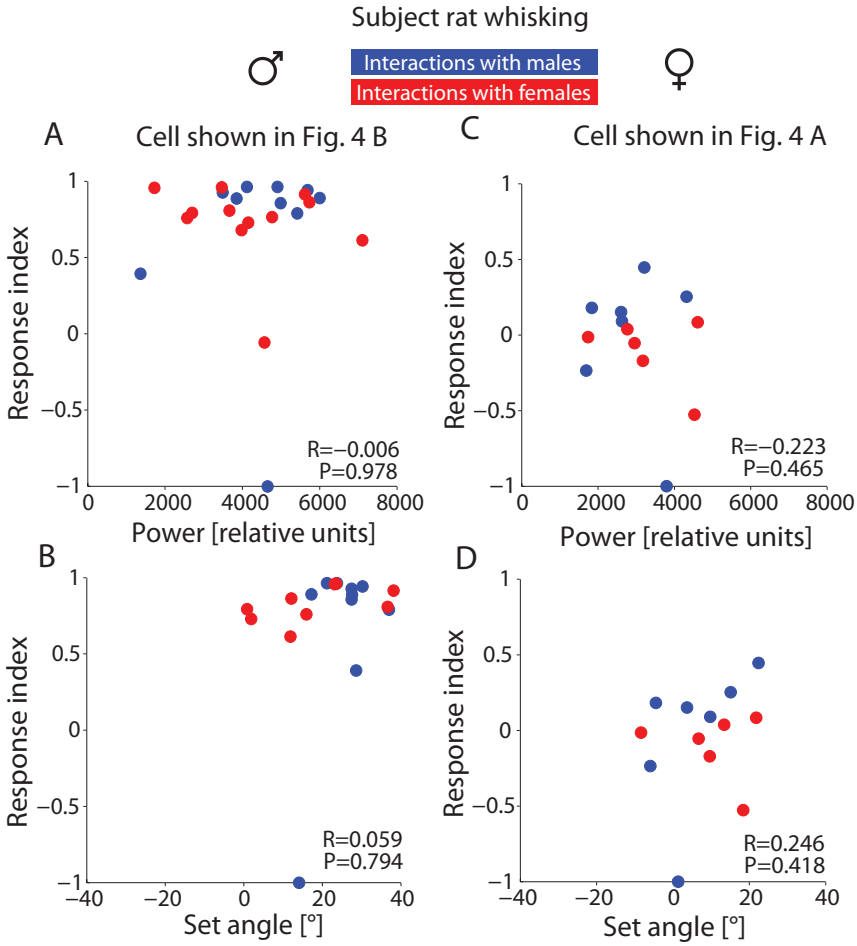



Figure 3.60: Subject rat whisking is only weakly and insignificantly correlated with neuronal responses in example cells. Interactions with male stimulus animals are represented by blue dots and interactions with females by red dots, respectively. A, Correlation of response indices from single interactions and whisking power for the SU in Fig. 3.56A,B, recorded from a male. B, Correlation of SU response indices from single interactions and whisker set angles. C,D, Correlations between whisking parameters and neuronal responses analogous to A,B, for the SU shown in Fig. 3.56C,D, which was recorded from a female rat. Numbers indicate Pearson's correlation (R) and the corresponding P-value (P).

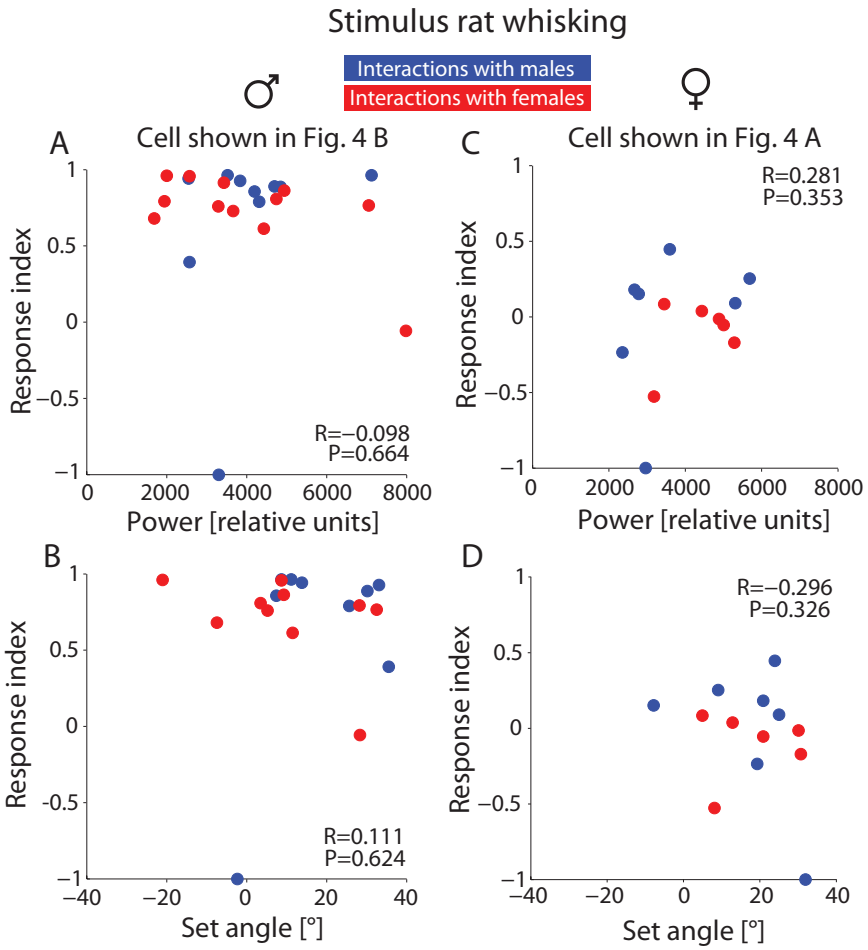



Figure 3.61: Stimulus rat whisking is only weakly and insignificantly correlated with neuronal responses in example cells. Same comparisons as in Fig. 3.60 for the relation of neuronal responses with whisking parameters of the stimulus instead of the subject rat. See legend of Fig. 3.60 for details.

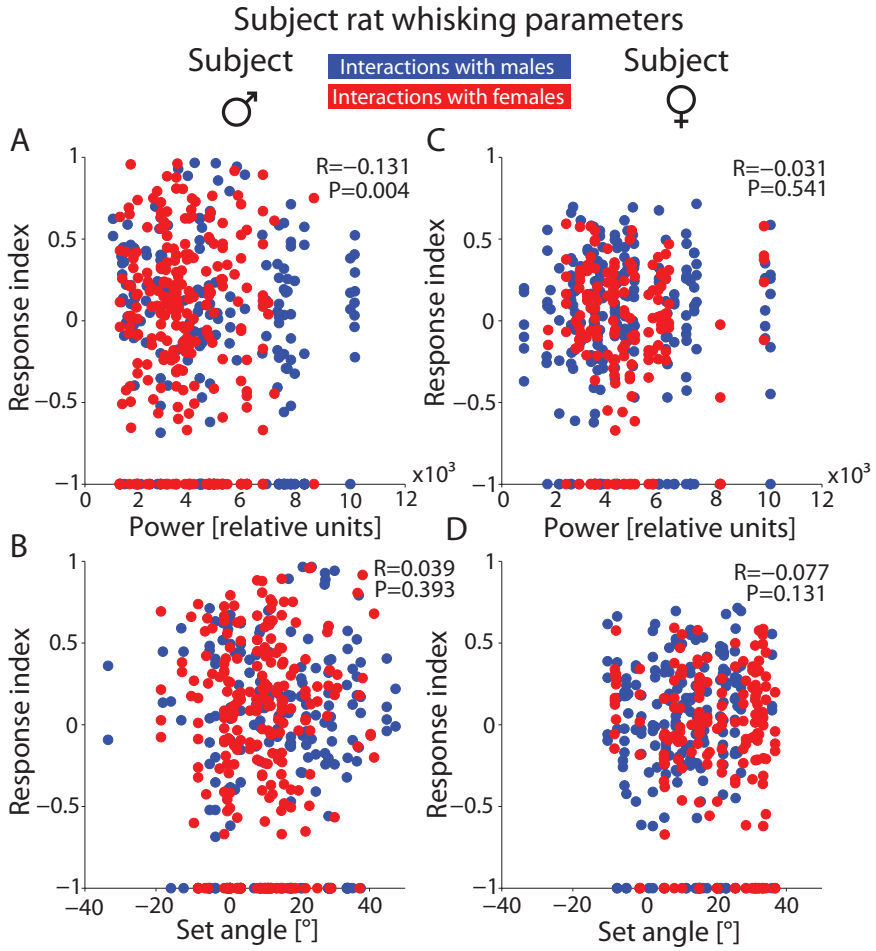



Figure 3.62: Subject rat whisking is only weakly correlated with neuronal responses across the population. Interactions with male stimulus animals are represented by blue dots and interactions with females by red dots, respectively. Data refer to 865 combinations of neuronal responses and whisking parameters. A, Correlation of response indices and whisking power for subject males, showing a weak but significant negative correlation. B, Correlation of response indices and whisker set angles for subject males. C,D, Correlations between whisking parameters and neuronal responses of subject females analogous to A,B. Numbers indicate Pearson's correlation (R) and the corresponding P-value (P).

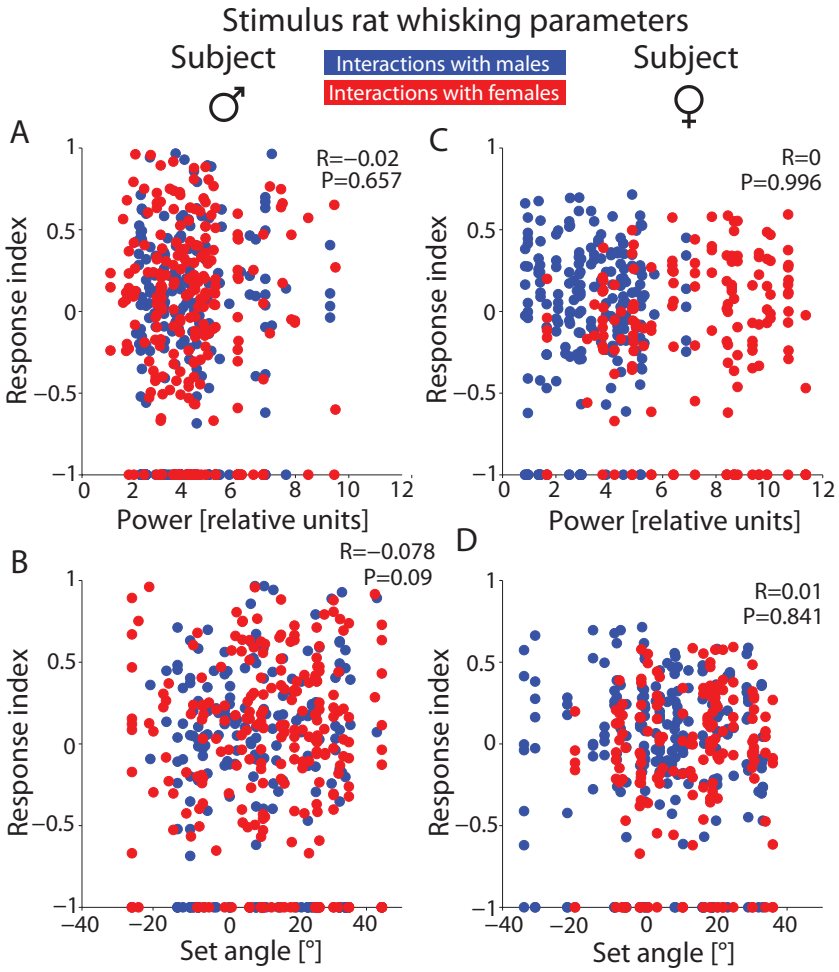



Figure 3.63: Stimulus rat whisking is only very weakly and insignificantly correlated with neuronal responses accross the population. Same comparisons as in Fig. 3.62 for the correlation of neuronal responses with whisking parameters of the stimulus instead of the subject rat. See legend of Fig. 3.62 for details.

# 4 Discussion

In the discussion I will follow the sequence found in the results sections. Thus, I will first focus on the methodological aspects of the work, including an assessment of the quality of clustering and of the recording stability, as well as the reliability of cell-type assignment. I will then discuss the behavioral observations, and proceed to the results from electrophysiological recordings, as well as behavioral results where they stand in direct relation to the physiology. Going beyond the observations made and results obtained in the present study I will then discuss in an outlook possible further methodological improvements, as well as analytical and experimental directions. The discussion ends with a concluding remark.

## 4.1 Methodological aspects

### 4.1.1 Spike clustering and assignment of single units

It has been shown that tetrodes (Wilson and McNaughton, 1993) are superior to single wires when spikes belonging to a certain SU are to be separated from other spikes (Gray et al., 1995; Harris et al., 2000). How tetrode recordings are to be analyzed, is, at the same time, a matter of disagreement. The first step is always the extraction of spike parameters like amplitude and half width. Then, however, some authors draw the borders between clusters manually, as described by Knierim (2002), while others use automated clustering algorithms, like the one developed by K. D. Harris ('KlustaKwik'), whose results can be further refined using 'MClust', developed by A. D. Redish. These algorithms make use of the multiple dimensions in which signals from a tetrode can differ, and thus have the potential to improve clustering relative to unassisted manual protocols (Harris et al., 2000). In addition, these algorithms allow for a quantification of cluster separation (Schmitzer-Torbert et al., 2005), although these separation values are also subject to error, and have only been validated quantitatively for hippocampal recordings. Because of the aforementioned advantages and the expertise in the lab (von Heimendahl et al., 2012), I decided to use an automated clustering algorithm. With regard to the inclusion criteria for separation quality, I chose a L-ratio threshold of 0.5, the upper of two L-ratio thresholds used by von Heimendahl et al. (2012). As spike shapes of neurons are more similar in cortex than in hippocampus (M. von Heimendahl, personal communication), and even neurons with compact clusters and clear refractory times sometimes had L-ratios above 0.2, this inclusion criterion seems sufficiently conservative.

### 4.1.2 Cell-type assignment and diversity of neuronal (sub)classes

There is disagreement between different authors as to whether spike shapes from extracellular cortical recordings can be used to reliably separate neuronal classes. However, already in the seminal study by Simons (1978), distinct extracellularly recorded spike shapes of RS and FS were described in BC. The landmark study with respect to the assignment of neocortical neurons to cell types based on extracellular features has been performed by Barthó et al. (2004). The authors recorded from a large number of L5 neurons and identified inhibitory

and excitatory neurons based on network interactions (i.e., inhibitory or excitatory effects on activity of other simultaneously recorded neurons)[6]. The authors then used the characteristic spike shapes of these two neuron types to classify the remaining neuronal population. Neurons could be assigned to two distinct clusters, characterized by narrow and wide spikes, respectively, quantified through peak-to-trough time and half width of the spikes. These correspond to putative FS and putative RS. The validity of the approach was further confirmed by the fact that neurons had always either excitatory or inhibitory influence on other neurons, never both. Importantly, the authors could show that neurons with narrow spikes were never excitatory, while a small subset (6%) of neurons with wide spikes was inhibitory. Finally, Barthó et al. (2004) also described bursting subtypes within both RS and FS, where the subdivision within RS could correspond to the one discussed in 4.3.4.

The criteria applied here are similar to those used by Barthó et al. (2004), but include post-positivity as an additional measure of spike shape (von Heimendahl et al., 2012). As described in 3.1.2, the distributions of these parameters were bimodal, which led to two distinct spike shape clusters. These two neuronal populations also differed in their firing rates, which supports their classification as putative RS and FS. A similar procedure for separation of SUs into these two classes has also been successful for primary auditory cortex (R. Rao, personal communication). Thus, the classification method applied here seems appropriate and it can be assumed that it assigns cell types correctly in most cases.

At the same time, some reservations should be kept in mind. First, the distinction by spike shape might not work equally well for all layers, such that the assignment was reliable for L5, but less so in other layers. Second, as already pointed out before, while FS are never excitatory, RS can also be inhibitory neurons. For example, Mateo et al. (2011) described a distinction between FS and non-FS GABAergic interneurons in L2/3, where the non-FS have wider spikes. Thus, the RS population should not be equated with pyramidal neurons, although there is broad overlap. Third, some neurons were observed for which the different spike shape features did not completely fit into any of the two classes. These were joined with the RS cluster, but their existence points to the underlying diversity within both pyramidal neurons (Chagnac-Amitai and Connors, 1989; de Kock and Sakmann, 2009; Jacob et al., 2012) and interneurons (Gupta et al., 2000; Porter et al., 2001; Nogueira-Campos et al., 2012; Perrenoud et al., 2013). Finally, the FS as described here have been classified exclusively based on their extracellular features. Thus, it is unclear in how far this is comparable to studies where the firing rate was used as a parameter for classification (Simons, 1978; Vijayan et al., 2010), and where, possibly as a consequence, much higher average firing rates of FS were found (18 Hz over different BC layers in Vijayan et al., 2010; a range of 15-50 Hz in Simons, 1978). In this context, it would also be interesting to apply the cross-correlation methods used by Barthó et al. (2004) to validate the classification based on spike shapes, which the authors also partly performed on data from tetrode recordings.

The fraction of FS was 23% in the present study, and thus much higher than the 9%

[6]Although data from S1 and prefrontal cortex were pooled, the conclusions can be expected to be representative of S1, being the recording location of 88% of analyzed neuron pairs.

reported by both Vijayan et al. (2010) and Barthó et al. (2004). Similarly low numbers were found by McCasland and Hibbard (1997), who reported ca. 10% of FS based on immunohistochemistry. In addition, a lower percentage of FS was also found in prefrontal cortex (8% reported by Jung et al., 1998) and primary auditory cortex (10%, personal communication from R. Rao). It is unclear, whether this is an effect of the distribution of recordings over layers, of different classification algorithms, or a combination of these and possibly other factors.

### 4.1.3 Tetrode positioning and recording stability

As described in 3.1.3, recording stability decreased, when tetrodes were moved immediately before the recording. This expected finding supports the view that instability in recordings was only partly due to changing unit activity, and was to a certain extent a recording artifact. The applied stability criteria, decribed in 2.3.4, were introduced to exclude unstable units. Based on these criteria, 45 or ca. 12% of SUs were excluded due to lack of stability. This might indicate that the applied criteria were relatively strict, but at the same time it also hints at the size of the problem. It should also be mentioned that the stability criteria applied were sensitive to the number of spikes, and instability in the activity of very sparsely responding units might have been missed more often. Overall, instability constitutes a potentially important confounder, and should be minimized whenever possible.

This could be achieved by displacing the tetrodes exclusively after recordings, so that at least one full day passes until the next recording. This was the dominating sequence of tetrode positioning in the last experiments, and was observed to improve stability. Further gains in stability might be made by leaving tetrodes in the same location even longer, but whether this had an additional positive effect on stability has not been quantified here, as tetrodes only rarely remained in the same location over subsequent days. It should also be mentioned that displacement of tetrodes before recordings was usually a reaction to low signal-to-noise ratios, leading to a low yield in recorded SUs. A more cautious strategy in positioning the tetrodes would thus increase stability, but decrease the number of recorded SUs.

A surprising finding was that amongst those instances, where the tetrodes were moved before recordings, a larger displacement was associated with higher stability. This was not a strong effect, and thus its relevance remains unclear. It can be speculated that it is based on the adhesive properties of neural tissue, such that the tissue relaxed only slowly for small displacements, while it was completely detached from the tetrode for large displacements, and thus found a new 'equilibrium' more rapidly.

### 4.1.4 Distribution of recordings over layers

The distribution of the respective unit types recorded in different layers is given in 3.1.4. Differences between the distibutions of RS and FS over layers were observed, but their relevance for the interpretation of the results remains unclear. Numbers are also reported split by animal sex, which is particularly relevant for the interpretation of the sex-specificity of

responses. Here, one specific observation is especially important with regard to the comparison of recordings from males and females. Thus, within the RS with verified layer, there was a high percentage of L5B units recorded from males (62%), while this layer contributed only 15% of RS in females. This constitutes a potential confounder when interpreting the results reported in 3.3.12, in particular the finding of higher modulation in male RS. At the same time, the finding that RS firing rates were in fact higher in females than in males argues against an overdominance of L5B, which is shown to have the highest RS firing rates. It is also important to keep in mind that the dataset with verified layer assignment constitutes only 60% of all RS. Thus, the amount of imbalance between L5B recordings from males and females in the overall RS population remains unclear. Finally, a comparison of L5B RS recorded in males and females indicated that even for neurons from a certain layer, sex-specific response differences were present (also see 4.3.11).

### 4.1.5 Determination of layer from recording depth instead of lesions

As described in 3.1.4, the recording depth as used in the presented analysis is insufficient to determine the laminar location of units with sufficient precision, taking the histological assignment as a gold-standard. For every transition between two layers there is strong overlap, and even a separation of L2/3 from L5A would produce many errors. This variability in recording depths for the same layers is to be expected for several reasons. First, the cerebral cortex is slightly curved at the location of BC, and thus the trajectory through layers is different according to the exact lateral coordinate. This is very clearly seen for the recordings from L4 in a more lateroposterior location within BC. Second, tetrodes are not always perfectly straight, which produces additional variability. It should be kept in mind, however, that some of the further sources of imprecision, in particular in determining the most superificial location where spikes were recorded, could be overcome by lowering the tetrodes more slowly at the beginning of experiments, and dedicating more attention to detecting and documenting this point. Besides, there are distinctive properties of different layers which can be used as additional qualifiers of a laminar location as gauged by depth. In particular, L2/3 neurons fired at much lower rates than L5B and other deep layer neurons, and L4 recordings were characterized by high background activity and difficulties in detecting SUs, probably due to the dense packing of small neurons in this layer (Welker and Woolsey, 1974; Ahissar and Staiger, 2010). Using recording depth, the firing properties of neurons, and the laminar assignment to nearby tetrodes should allow to probabilistically assign coarse laminar location (supragranular/granular/infragranular) to units without corresponding histological lesions. The precision of this assignment would be naturally inferior to the one based on lesions, but could be sufficient to justify the definition of a wider dataset, on which hypotheses with regard to cortical layers, as generated based on the higher-quality dataset, could be additionally tested. This method could potentially be validated by dividing the set of units with histologically verified laminar location into a training and a testing dataset.

## 4.2 Behavioral results

### 4.2.1 Interaction patterns as a function of rat sex

As described in 3.2.1, interaction patterns differed in several ways between males and females. The strongest effect observed was the female preference to interact with males, and the lack of a corresponding sex-preference in the interactions of male rats. This reproduces the observations of Wolfe et al. (2011), who found the exact same pattern. In addition, it was shown here that the female preference to interact with males is independent of the estrus state of the female. It can be speculated that the equal amount of interest that male rats dedicate to male and female conspecifics reflects the involvement of males in both sexual and dominance relationships. The latter might make it necessary for a male rat to monitor the males in its environment, while a similar motivation of female rats to observe other females might be absent. However, even if this hypothesis were correct, it would not explain why the overall interaction times of females are so much higher.

Significant differences also emerged in the latencies to interact. The observation that females approached males faster during estrus, when they are sexually receptive, was in line with the expectations. The reasons for the much, although not significantly, shorter latencies to interact for male as compared to female rats are not so easily explained, however. Fear might be a factor which contributes to longer latencies in females, but this would suggest longer latencies to interact with stimulus males primarily, which was not the case.

### 4.2.2 Comparison of interactions with animate and inanimate stimuli

To characterize BC activity during social touch, and in how far it is different from other types of touch, responses to control stimuli were observed, and to be able to interpret these, in turn, it was necessary to describe interactions with animate and inanimate stimuli on the behavioral level. For objects as stimuli, these behavioral observations stem exclusively from the same experiments, as those, where neuronal recordings were obtained, as described in 3.2.4. For the stuffed rat as stimulus, however, there was an additional separate set of purely behavioral experiments, as described in 3.2.3, which was free of some of the biases present during physiological recordings.

The overall pattern of observations suggests that stuffed rats elicited the least interest, and alive rats the most. Objects were rather like alive rats by most measures. In the purely behavioral experiments it was shown that stuffed rats were approached at longer latencies than alive rats, and were interacted with less often. The additional measure of time spent close to the different stimuli also revealed a very strong preference of alive over stuffed rats. These findings were largely corroborated by patterns of interaction with the stuffed rat during physiological recordings. Most importantly, the time spent in interactions was again longer for alive than stuffed stimuli.

For objects as stimuli, the picture was less clear. Thus, neither was the latency to interact with objects higher nor was the overall time spent touching them lower than the corresponding measures for alive rats. The longer average duration of single interactions with objects than

alive rats indicates, however, that differences were present in the way these two stimulus types were treated.

Although the presented data might suggest that the overall level of interest was not different between alive rats and objects as stimuli, qualitative observations made during experiments suggest that this might have still been the case. For physiological experiments, the comparison of interactions with alive rats and inamimate stimuli is complicated by the mode of presentation. Most importantly, the strategy applied to maximize the number of interactions and keep the rat interested included a shortening of stimulus presentation, when no interest at all was shown. This was much more often the case for objects and the stuffed rat, and thus there is an experimental bias against long periods of inanimate stimulus presentation devoid of interactions.

Two further factors might have aritificially increased the observed interaction times with inanimate stimuli: First, they were presented alone and not in a chooser setting. The behavioral experiments with stuffed rat presentation in a chooser setting show the decreasing effect that the presence of an alternative alive stimulus exerts on the interest in the stuffed rat. Second, objects, but not the stuffed rat, were new in most sessions. This novelty factor might have contributed to increasing the interest in objects. This might also explain why latencies to object touch were considerably, although not significantly, lower than even to the touch of alive rats. The latency comparison, however, should be interpreted with additional care, because high interest in one animal was bound to produce long latencies for the other stimulus in a chooser setting, an effect not present for inanimate stimuli.

### 4.2.3 Whisking onto animate and inanimate stimuli

While the behavioral preference to interact with alive rats was very pronounced compared to the stuffed rat, but not so clear compared to objects, both inanimate stimuli were similarly different from alive rat interactions with regard to whisking behavior. The subject rats investigated both objects and the stuffed rat with highly regular, high-amplitude whisks from relatively protracted set angles, as detailed in 3.2.5. Whisking onto alive rats, in contrast, was much less regular and whiskers were held more retracted on average. These differences in whisking behavior might underlie the differences in neuronal responses to the touch of animate and inanimate stimuli, as discussed in 4.3.10.

The question remains open, in how far the decrease in amplitude and regularity during alive rat interactions is caused by an active change in sampling strategy. An alternative, although not mutually exclusive, hypothesis would be that the whisking of the subject rat was passively disturbed by the physical resistance of the stimulus rat head and/or whiskers. This could in particular account well for the higher irregularity of whisker movements during social interactions. It is conceivable that the active sampling of immobile stimuli is optimized in line with the minimal-impingement strategy (Mitchinson et al., 2007), but this breaks down during the sampling of unpredictably moving stimuli as alive rats. In addition, olfactory information might be collected during alive rat touch. This might contribute to smaller distances between

the subject rat head and alive stimuli, as the rat is trying to come closer to the cheek glands which are a source of pheromones. This would be expected to lead to different head-to-stimulus distances for different stimuli. In addition, if the whisking is passively disturbed by the presence of the stimulus rat's whiskers, set angles and whisking amplitudes of the subject rat should be more similar to the touch of inanimate stimuli when the stimulus rat's whisker were trimmed.

## 4.3 Physiological results

### 4.3.1 Barrel cortex population responses to social touch

Many neurons were observed to change their firing rates significantly during social interactions, as described in 3.3.3. Excitation was more common, but there were also instances of inhibition. Overall, 38.4% of RS and 47.2% of FS changed their responses significantly at a significance level of 0.05. These numbers leave no doubt that social touch was reflected in the neuronal activity of both RS and FS on the population level.

Studies investigating the response properties of BC neurons to more conventional stimulation repeatedly reported higher percentages of modulated units. Thus, Simons (1978) reported that 90% of BC units showed a distinct somatosensory stimulus preference. The number of significantly modulated units reported here is much lower. There are several factors which might have contributed to this, and which suggest that the percentage reported here is a lower-bound estimate, and probably a severe underestimation. First, Simons (1978) showed 58% of units to be phasically responsive to stimulus transients. The timescale on which the present analysis was performed does not resolve such response transients, and if excitatory-inhibitory responses, as also described by Krupa et al. (2004), have been present, these might have been missed. The presented analysis has been conducted in the framework of rate coding, and possible temporal codes for stimulus-related information have not been assessed. Second, as interactions were not rewarded or even enforced, the number of interactions was limited by the intrinsic interest of subject and stimulus animal. Thus, on some days, there were only few social interactions observed. At the same time, the probability of finding a modulation to be significant is decreasing with the amount of time spent in interactions. This is aggravated by low firing rates in some neurons, as shown by the lower probability to find significant modulations in low-firing units. In fact, some low-firing units seemed to exhibit modulations on visual inspection of PSTHs, without this modulation reaching significance threshold. Third, it should also be noted that it could not be observed whether the whisker(s) corresponding to a specific neuron's RF were touched, and if they were, at what point in time this was the case, and with what strength. The exact bending moment which transduces whisker touch to the follicle and ultimately modulates response strength (Jadhav et al., 2009; Pammer et al., 2013) was not known. This does not influence the probability of finding a specific neuron's modulation to be significant, but it leaves open the possibility that social touch perception occurred through other whiskers than the one corresponding to a recorded neuron. Finally, some units might not have responsed strongly because their specific tuning was not matching

the distribution of stimulus velocities and/or angles present during social touch.

Response increases during social touch were larger in FS than RS, although not significantly. This difference was in part due to the absence of strongly inhibitory responses in FS. The finding of more robust and mostly excitatory responses of FS is in line with the work of McCasland and Hibbard (1997), who compared metabolic activity in GABAergic and non-GABAergic hamster BC neurons, and observed higher touch-related activity increases in GABAergic neurons. The authors suggested that the two compared cell types correspond to FS and RS, respectively. Stronger responses of FS are also to be expected from the findings of Simons (1978) and others who showed that FS respond more reliably to touch and have a broader angular and frequency tuning (reviewed by Swadlow, 2003). It is, however, not established that response modulations of FS are consistently higher in relative and not just absolute numbers, at least for awake behaving animals.

### 4.3.2 Layer-dependence of regular-spiker baseline firing rates and detection of low-firing neurons

With regard to the use of the terms 'spontaneous' or 'baseline' firing rate, it should be noted that in many studies, in particular for head-fixed animals, spontaneous firing rates are reported at quiet rest, while in the present study the baseline firing included all time periods except for the times of interaction. Thus, the 'baseline' included sleeping, quiet wakefulness, grooming, running, free whisking, and touch of the floor and walls. Consequently, the baseline firing rates reported here should be taken as approximations. For a better comparability with previous studies, behavioral scoring during the complete recording sessions and subsequent restriction to the times of display of certain behaviors would be necessary.

**Layer1** No L1 neurons could be recorded, and the number of MUs was also very low. The reasons for this are presumably both anatomical and methodological. First, removal of the dura might have damaged or temporarily disturbed superficial neurons. Second, directly after surgery, tetrodes were located where the first spikes were recorded. This was done to already position the tetrode in the cortex and avoid supracortical bleeding or dura regrowth from damaging the tetrode during a later penetration. Third, apart from spontaneous firing, this spiking activity was determined as a response to whisker touch. At the same time, L1 neuronal density is low (Zhou and Hablitz, 1996), and L1 seems to be rather involved in top-down modulation of signals than bottom-up signal representation (Letzkus et al., 2011). This would presumably lead to these neurons being particularly silent in anesthesia, while ketamine anesthesia through its inhibitory action on NMDA channels (Franks, 2008) already attenuates overall neuronal activity. Taken together, the method of tetrode positioning and the properties of L1 neurons presumably combined to render recording from L1 very rare.

At the same time, it can be speculated that top-down modulation plays a crucial role in generating the sex-specific responses reported here, and this might be partly mediated by L1. If, alternatively, subtle motor differences which were missed by the present analysis were the basis of sex-specific responses, correlates of these would presumably also be detectable in L1,

as the influence of M1 on BC computations, in particular by L5 cells, is known to be mediated by synapses formed in L1 (Xu et al., 2012). Thus, it would be interesting to record activity in L1 during social touch using other methods than tetrodes, in particular two-photon imaging.

**Layer 2/3** Neurons in L2/3 are known to fire at very low rates. Patch-clamp recordings have repeatedly shown mean firing rates of L2/3 neurons to be under 1 Hz. Thus, Brecht et al. (2003) report an average spontaneous firing rate of 0.07 Hz under urethane anesthesia, de Kock and Sakmann (2009) found an average rate of 0.31 Hz in quiet awake rats, and the average rate during quiet wakefulness in the studies of Poulet and Petersen (2008) and Crochet et al. (2011) using mice was 0.61 Hz and 0.2 Hz, respectively. This is in line with the findings of Kerr et al. (2007), who, employing two-photon imaging in mice, showed L2/3 neurons to fire with 0.25 Hz on average. Both patch-clamp and two-photon imaging do not have an inherent bias to sample highly active neurons, as has been proposed for extracellular electrode recordings. However, the neuronal firing rates found in L2/3 in this study put a supposed bias towards sampling an unusually active neuronal population, when recording with tetrodes, under question. For a population of 23 L2/3 RS, average baseline firing rate was 0.22 Hz, and thus in the same range as observed with intracellular methods. Although this does not preclude a bias, it indicates that in this study neurons with low firing rates could also be recorded, and that a bias, if present, was not very pronounced. This is supported by the example of a low-firing L5B RS which displayed very sparse interaction-related activity, as shown in Fig. 3.17B.

**Layer 4** The activity of L4 neurons reported here was also surprisingly close to firing rates recorded intracellulary. The first intracellular recordings in L4 of BC were performed under urethane anesthesia and indicated extremely low spontaneous firing rates of 0.05 Hz on average (Brecht and Sakmann, 2002). However, in a later study which compared firing in the awake and anesthetized states, mean firing rates in L4 were found to be 1.93 Hz in the awake state, and thus much higher than the 0.58 Hz observed during anesthesia (de Kock and Sakmann, 2009). These 1.93 Hz are very close to the 2.08 Hz mean firing rate observed here for L4 RS. As has been the case for L2/3, this argues against a strong bias to detect high-firing neurons due to deployment of tetrode instead of intracellular recordings.

**Layer 5** The mean spontaneous firing rates observed in this study were 2.77 Hz for L5A and 3.03 Hz for L5B. The finding that L5 units fire at higher rates on average than units from other layers (see Fig. 3.30A), is supported by the literature. The precise firing rates reported, however, differ widely. In a patch-clamp study by Manns et al. (2004) in anesthetized rats, spontaneous firing rates were higher in L5B (mean 0.77 Hz) than in L5A (0.39 Hz). Stunningly, Jacob et al. (2012) also using patch-clamp recordings under anesthesia report average firing rates of 10.6 Hz for L5-RS and 11.8 Hz for intrinsic bursting cells (for details on the subtypes see 4.3.4). The rates reported by de Kock and Sakmann (2009) are in between these (1.62 Hz for slender-tufted and 4.12 Hz for thick-tufted pyramidal neurons), also recorded under

anesthesia. Thus, the responses observed here are within the range described by other authors using intracellular methods. In addition, as described in more detail in 4.3.4, the slightly higher firing rates observed in L5B than L5A are also in line with evidence on different anatomical and physiological cell types, and their distributions between the two sublaminae.

**Layer 6** There is relatively little data available in the literature on the firing rates of BC L6 neurons. The baseline firing rate of 1.79 Hz observed here was higher than the 0.52 Hz described by de Kock and Sakmann (2009) in awake head-fixed rats. However, the population of RS recorded in L6 in the present study was very low (n = 6), and thus an assessment of firing rate is difficult. Interestingly, in this layer more FS (n = 9) than RS were recorded. It is unclear, whether this is chance or reflects the relative percentage and/or firing properties of L6 RS and FS. Apart from such possible differences within the L6 neuronal population, the generally low number of SUs recorded in L6 might be due to two methodological factors. First, recording quality decreased over the course of experiments, and thus in many cases it was difficult to record SUs in the end of experiments, when L6 had been reached. Second, as responses were particularly strong in L5B, there was an incentive to move slowly in this layer, correspondingly taking longer to reach L6.

**General remarks** The use of tetrodes has been repeatedly claimed to introduce a bias towards sampling of highly active neurons (Olshausen and Field, 2004; Chorev et al., 2009). However, although this assumption seems reasonable and receives indirect support from many studies, there is little direct evidence. While studies supporting the extracellular recording bias do exist (Towe and Harding, 1970; Stone, 1973), they are methodologically in many ways incomparable to the tetrode/patching distinction in question. As pointed out before, many studies do support lower firing rates when neurons are recorded intracellularly, but others do not. This question can be answered through comparison of firing rates obtained using intra- and extracellular methods. Ideally, this would be tested on the same cells using both recording methods, but if this is not the case, the cells to be compared have to be minimally in the same brain area and layer, as well as of the same cell type. Barrel cortex would seem a uniquely well-suited area to settle this question, due to the rich literature accumulated on different aspects of its physiology, but the diversity of protocols does not permit a final judgement on how strong this bias is in which layer, and in fact whether it exists at all. These distinctions between protocols include awake vs. anesthetized animals[7], with different anesthesia protocols further complicating the picture, freely-moving vs. head-fixed animals, and different sampling over layers, which is not always reported (e.g. by Vijayan et al., 2010). In addition, both extra- and intracellular recording methodologies can differ, as do

[7] In an early work, Armstrong-James and George (1988b) described decreasing BC activity during anesthesia. The strongly damping effects of anesthesia on BC activity were reproduced by Vijayan et al. (2010) using tetrode recordings during isoflurane anesthesia. They observed an average firing rate decrease from 6.1 Hz in the awake state to 0.15 Hz during anesthesia. While >75% of RS fired with rates >1 Hz while awake, the same neurons always fired at <1 Hz in anesthesia. To my knowledge, such a comparison is not available for the effects of urethane anesthesia, presumably because of its irreversibility.

e.g. single-contact tungsten microelectrodes and tetrodes. To name one example, Curtis and Kleinfeld (2009) recorded in rat BC with stereotrodes and observed a much higher average firing rate of 9 Hz. What is more, the authors hardly observed any units with a firing rate of <5 Hz. As they recorded in all cortical layers, the discrepancy in rates between this and the present study, as well as the very high number (>50%) of neurons with narrow spikes (see Fig. S6 in Curtis and Kleinfeld, 2009), is presumably indicative of discrepancies in the sampling properties of different extracellular recording methods and protocols.

As described above, neurons with very sparse interaction-related responses, as well as firing rates down to 0.05 Hz, were recorded. The comparison of the observed RS baseline firing rates with the literature not only confirms strong layer-specific differences, but also indicates that absolute values are at least close to those obtained by intracellular recordings. This does, of course, not preclude bias completely, and in particular the practice of repositioning the tetrode if no spikes were detected could potentially have contributed to missing very silent and/or very sparsely responding neurons.

### 4.3.3 Layer-dependence of fast-spiker baseline firing rates

The population of 72 FS was relatively small, when split by layers. In addition, there are several inhibitory neuron types, some, although not all of which are FS. Probably due to these complications, there is also only little data in the literature to compare the presented results with, as only few studies have at the same time distinguished FS from RS, recorded a sufficient number of FS, and assigned layers to recordings, in particular in awake animals.

In a detailed study of the intracellular response properties of L2/3 interneurons, Gentet et al. (2012) reported very high mean firing-rates for FS interneurons (9.4 Hz). The other interneuron types in L2/3 were reported to have average baseline firing rates of 3.7 Hz (non-FS) and 6.3 Hz (somatostatin interneurons). Thus, L2/3 FS were firing by at least one degree of magnitude more than average excitatory neurons, which displayed rates of 0.4 Hz. This is in stark contrast with the baseline rates observed here. Of ten units classified as L2/3 FS, nine had very low average rates with a mean of 0.3 Hz, being very similar to the rate found for units classified as RS. Only one FS fired at a very high rate of 23.7 Hz. It is unclear, how these findings might be reconciled. Clearly, the dataset reported here is small, but nevertheless the firing rates are so hugely different that it might be doubted whether the units classified as L2/3 FS were not in fact RS. The methodological differences, however, do not permit a direct comparison.

As reviewed by Gentet (2012), L2/3 interneurons also have diverse functions, including disinhibition, dendritic gating, and the control of state changes. The finding of comparatively high response indices in L2/3 FS indicates that these do not belong to the somatostatin neurons, which decrease their activity during active whisking (Gentet, 2012), but further assumptions can hardly be made.

With regard to other layers, there is a lack of studies on the functional properties of BC interneurons. Koelbl et al. (2013) show that within the group traditionally classified as FS in

L4, there are different neuronal subtypes. There are further studies of interneuron subtypes in BC, but they have all been performed in slices (Ma et al., 2006; Karagiannis et al., 2009; Staiger et al., 2009). Thus, the observation of low firing rates in L5A FS and very high firing rates in L5B and L6 FS seems to be the first report of firing rates of infragranular layer FS in the BC of awake behaving animals[8]. However, the low number of FS per layer, in particular for L5A (n = 5) does not allow for a thorough quantification, and the identification of FS using extracellular data remains putative.

### 4.3.4 Layer-dependence of response strengths

**Layer 2/3** The low firing rates of RS in L2/3 are associated with weak overall modulations as well (Fig. 3.33). The population PSTH shows that there is an average response increase of L2/3 RS after interaction onset (Fig. 3.32), which is, however, only transient, so that it does not translate into a strong overall modulation during interactions. This might be a consequence of the fast decay in L4 RS responses (see next paragraph), through the strong projections from L4 to L2/3 (Shepherd and Svoboda, 2005). The weak responses observed here are in line with the very low number of spikes evoked by deflections of even the principal whisker (0.031 spikes/deflection; Brecht et al., 2003). Using calcium imaging, O'Connor et al. (2010a) observed very sparse touch representations in L2/3, and even the few robustly activated neurons had very low event rates of under 0.15 Hz. However, the same authors reported a much higher mean firing rate of 3.0 Hz in response to touch for their intracellular recordings (O'Connor et al., 2010a). These two findings might be reconciled by the observation that although the mean firing rate in intracellular recordings was much higher, the median of stimulus-evoked firing rates was 0.2 Hz, and thus in the range of calcium events. In extracellular recordings, Krupa et al. (2004) found that during active tactile discrimination, L2/3 neurons were firing at a rate of 7.0 Hz, and often showed sharp response peaks. This is in stark contrast to the touch-related firing rates reported here (mean 0.24 Hz). The example units presented by Krupa et al. (2004) indicate, however, that the authors sampled a particularly active population of L2/3 neurons. The subset reported here might have a smaller bias towards highly active and, at the same time, highly modulating units. But it should also be kept in mind that the responses reported here include the complete interaction period, and are not restricted to shorter periods of strong stimulus-locked reponses. In addition, the stimulation during social touch was in no way optimized to engage the whisker corresponding to the RF of recorded cells.

**Layer 4** Responses of BC during active sensing have been shown to be less sustained in L4 than in L5/6 (Krupa et al., 2004). Similarly, Curtis and Kleinfeld (2009) observed that slowly-excited units are rare in L4, as opposed to both supra- and infragranular layers. In addition, RFs of L4 have been shown to be small, with 85% of units responding to the deflection of the principal whisker only (Simons, 1978). These two factors could explain

[8] Barthó et al. (2004) recorded L5 FS in freely-moving animals, and thus must have obtained data on firing rates as well, but these seem to have remained unpublished.

why the observed response modulations were relatively weak in L4. Firstly, responses were analyzed on timescales of hundreds (as in the PSTHs) or even thousands of milliseconds (as in the calculation of indices for complete interactions). Secondly, the spatial distribution of whiskers stimulated during an interaction also presumably strongly varied as a function of approach angle and velocity, whisker set angle and amplitude, and nose distance. Thus, the stimulus might have been spatially suboptimal for driving L4 neurons, and if a short-latency sharp stimulus-driven activity component in L4 was present with regard to single whisker touches, it might have decayed too rapidly to appear in the PSTHs. For a temporally and spatially very precise stimulus, one might have expected a sharp peak in the PSTH after stimulus onset, but this was presumably precluded in this case by the imprecision introduced through the inference of whisker touches from interaction onsets. While these were defined as the times of first overlap between any whiskers, the time points when certain whiskers relevant for the respective neuron's activity were touched, if at all, could not be determined.

A neuronal substrate for fast feedback mechanisms which could mediate rapid response offsets in L4 was described recently (Koelbl et al., 2013)[9]. In addition, Krupa et al. (2004) also showed L4 to contain a particularly high number of neurons which display multiphasic responses. Again, the interaction-based rather than stimulus-based analysis might have made it impossible to capture more complex response dynamics, with the consequence of no detected response changes, where actually excitation and inhibition took place sequentially.

**Infragranular layers** Infragranular layers are not accessible by two-photon imaging of calcium signals, or at least have become accessible only recently (Andermann et al., 2013). Thus, the number of studies which target deep layers is smaller than for superficial layers, in particular in awake animals. It has been shown repeatedly, however, that infragranular layers have large RFs compared to other layers (Armstrong-James and Fox, 1987; Zhu and Connors, 1999; Manns et al., 2004; de Kock and Sakmann, 2009). The finding that RS responses during interactions were strongest in L5B is in line with this, as the stimulus in social interactions comprises touch over an array of several whiskers, and thus neurons which summate over several whisker inputs would be expected to be more strongly excited.

The callossal inputs to BC, which relay information about ipsilateral whisker touch, are particularly strong in L5, and are producing a net inhibitory effect (Shuler et al., 2001). This would presumably apply to social touch, as interactions are typically symmetrical and the ipsilateral whisker pad is also stimulated. However, as the stimuli and their time course were very different between the present study and Shuler et al. (2001), it remains unclear whether interhemispheric projections also dampen L5 responses to social touch.

The observation that L5B RS were significantly more strongly excited than RS from L5A indicates that within infragranular layers, distinctions with regard to response properties exist, as suggested by the literature. Thus, the evoked responses observed by Manns et al. (2004)

[9]The authors show a subtype of parvalbumine FS interneurons, whose axons are restricted to L4 in barrels, and which are reciprocally connected with spiny neurons. Thus, they could serve as a feedback mechanism to reset BC activity after sensory activation.

in anesthetized rats were similar in L5A and L5B, but they observed larger RFs in L5B. The latter might help to reconcile their observations with the reported finding that L5B had stronger response modulation than L5A (see Fig. 3.33), as the touch during social interactions presumably involves many whiskers weakly, while Manns et al. (2004) stimulated the principal whiskers strongly. However, it should also be noted that Zhu and Connors (1999) did not find a gradient of RF size with depth within L5.

While Manns et al. (2004) did not distinguish subtypes of L5 pyramidal neurons, others have described two distinct classes of pyramidal neurons in L5 (Chagnac-Amitai et al., 1990; Schubert et al., 2001; Jacob et al., 2012). These two types are labeled intrinsic-bursting (IB) and regular-spiking neurons, where it should be noted that in the nomenclature used throughout this work, both would be termed RS, as opposed to FS. Thus, the regular-spiking type of L5 pyramidal neurons is referred to as L5-RS. While L5-RS are found in both L5A and L5B, IB neurons are much more prominent in L5B (Jacob et al., 2012). Using distribution and reconstruction data, Jacob et al. (2012) concluded that L5-RS and IB neurons are likely to correspond to slender-tufted and thick-tufted neurons (Larkman and Mason, 1990; de Kock and Sakmann, 2009), respectively. Although the mapping of physiology onto anatomy is not yet achieved, and in some regards these two neuronal types might be two extremes of a continuum, there seems to be a wide consensus that L5B contains a particularly high number of IB-type pyramidal neurons, that these have large RFs, and, in agreement with this, seem to integrate excitatory inputs across different columns, while L5-RS are involved in intracolumnar processing (Schubert et al., 2001). Considering the diffuse nature of stimulation during social whisking, the stronger modulation of L5B cells is thus in line with the literature.

The data reported here have not been analyzed with regard to the exact bursting properties of neurons. Although the information that can be gathered by extracellular recordings is limited, the literature suggests the hypothesis that bursting patterns could be used to distinguish these two subclasses of pyramidal neurons. If this were the case, IB neurons would be expected to respond differently, and probably more strongly, than L5-RS. It should be noted that within L5-RS, a further distinction between two subtypes is made by some authors (Chagnac-Amitai and Connors, 1989) based on intrinsic properties of neurons determined in brain slices. Whether these subtypes show different functional properties in behaving animals, is unknown.

In the context of relatively weak L4, but strong L5B responses during social touch, it is also interesting to note that L5/6 have been shown to receive direct thalamic input with latencies comparable to L4 (Constantinople and Bruno, 2013). These projections might contribute to the activity of L5/6 neurons being much more independent of L4 activity than has been thought previously (Constantinople and Bruno, 2013).

With regard to L6 neurons, it is difficult to draw any conclusions on a population of just six recorded RS, as pointed out before. Their responses seem to be close to average in rate and variability, as well as in the modulation, as measured by response indices. Overall, there seems to be limited knowledge and no consensus yet on the precise roles of L6 RS in BC. The relatively weak response modulations observed here are in line with Hooks et al. (2011), who

report that excitatory projections to and from L6 are weak in BC.

**Fast-spikers** Response modulations of FS were stronger in L2/3, L4, and L5B than in L5A and L6. These differences were not significant, however, and overall the sample of FS might have been too small to observe distinctive patterns. The mentioned variability of interneuron types might contribute to this additionally. Layer 6 FS (n = 9) seem to be a particularly active and variable population of neurons on average, but were rather weakly modulated by social interactions. The observed variability of L6 FS firing rates might reflect the distinctive property of L6, its high anatomical variability with respect to both inhibitory and excitatory cell types, as reviewed by Briggs (2010).

As discussed before, there are few reports of FS activity in awake behaving animals in the literature, especially with an attribution of cortical layer, and the specific interneuron types recorded in the present study are unknown. Thus, the only conclusions that can be drawn are that the layer distribution of interaction firing rates of FS is not strongly different from the pattern observed for baseline firing rates, and that no layer seems to show a homogenously strong response change in one direction.

**General remarks** Overall, the response strength on the population level reported here might seem low in comparison to the literature (Krupa et al., 2004; Hentschke et al., 2006). Response strengths are not directly comparable, however, because of the different windows and timescales in this compared to most other studies of BC sensory responses. Although this study uses an event-related design, the event to which interactions are aligned is interaction onset or head touch, while the actual time point of whisker touch remains unknown. Thus, an analysis of a time window of $<$200 ms after a certain stimulus is given, is not possible here, and the precise time when the neuronal response occurs is distributed over a longer period. The effect is a 'blurring' of responses, as can be seen in the slow increase of responses after interaction onset in the PSTHs. This is aggravated by the fact that the beginning of interactions was determined for the whole whisker pad, while responses of individual neurons will depend on when the whisker corresponding to this neuron's RF was touched.

Another important aspect which might contribute to small overall responses and is associated with the timescale of analysis is late inhibitory components of the response. Since Carvell and Simons (1988) observed post-stimulus inhibition in awake paralyzed rats, it has been repeatedly shown that many BC units exhibit a multiphasic response where typically excitation is followed by inhibition (Delacour et al., 1990; Krupa et al., 2004). On the analyzed timescale of hundreds of milliseconds to seconds, such dynamics are not captured, and thus the performed analysis works on the assumption of a very slow rate code. The ideal way to overcome this would be to determine the exact times and strengths of whisker touches, but there is currently no feasible way to do so. As an approximation, an estimate of these parameters could be attempted by taking into account borderline conditions like head distance along different axes, head velocity and acceleration, and the phase of subject and stimulus rat whisker motion, all of which can potentially be obtained by automated tracking of videos

filmed from different angles.

### 4.3.5 Response timing as a function of receptive field location

The observation that the units with RFs on posterior whiskers increased their firing earlier when the two rats approached each other at the beginning of interactions (see 3.3.5), is opposite to the assumption that the anterior whiskers would be the first to have contact with the stimulus (Sachdev et al., 2001), and thus the neurons with corresponding RFs would be the first to respond. Somewhat confusingly, the expectation is met at interaction offset, where units with anterior RFs are responding strongly, when the 'posterior' ones have already decreased their firing. One possible way to reconcile these observations is the interplay of whisker lengths and the development of whisker set angles over the course of interactions. The posterior macrovibrissae are longer and sample a much larger area in space than the anterior macrovibrissae (Brecht et al., 1997). At the same time, the whiskers are held more protracted at interaction onset (Fig. 3.38). This more protracted whisker position could lead to the effect that the posterior vibrissae came in contact with the stimulus rat earlier, which in turn could have led to the earlier activation of neurons with corresponding posterior RFs. In contrast, at interaction end, whiskers were held more retracted, such that when the rats separated, the anterior vibrissae could have been the last ones to be touched, and consequently the corresponding neurons could have been the last ones to reach their response minimum.

Importantly, it should be kept in mind that the tracked periods are not identical with interactions. On the one hand, an approach period of 100 to 200 ms was sometimes included in the videos used for high-speed tracking, which would have been considered still outside of the interactions in the scoring of events based on low-speed videos. On the other hand, tracking was sometimes made impossible by occlusion, and, as a consequence, some periods of whisker tracking started and/or ended in the middle of interactions as determined by low-speed video analysis. There are additional important constraints, which complicate the analysis of such correlations between RFs and response timing: the timing of interaction events only has low-speed video precision ($\pm$33 ms), the time points of exact whisker touches are unclear, and RF mapping in awake animals is imprecise. However, the observations of Sachdev et al. (2001) support the view that at least the temporal precision might be sufficient, albeit suboptimal. The authors report that the touch of a piezo-contact-sensor was delayed by 24 ms between a given whisker and the next whisker from the same row, which could be resolved by a frame rate of 30 Hz in most cases. In addition, this delay can be assumed to increase further for more distant whiskers along one row. Overall, despite constraints regarding the exact timing of touch, the behavioral and neuronal data reported in 3.3.5 indicate that with the used methods it is possible to extract data on the fine temporal structure of interaction-related neuronal responses.

### 4.3.6 Head positioning during interactions as an optimization strategy

As rats were unrestrained, their positions during interactions varied over the width of the platform and the space over the gap which was accessible to the rats from either side. Thus, to average across interactions in a meaningful way, head-centered interaction and firing maps were computed for a subset of the data. To this end, the positions of the nose of the stimulus rat and the nose positions where spikes were evoked were rotated to and replotted in a coordinate system centered on the nose of the subject animal (see 2.7.3 for details). This allowed to observe patterns of relative rat position during interactions, as well as response fields of BC neurons during social touch.

Rats were found to spend most of the time in interactions in close facial contact. This is in line with the finding of close object contact with microvibrissae, as observed during object discrimination tasks (Brecht et al., 1997), as well as during the investigation of unexpectedly appearing objects (Grant et al., 2012). As described by Brecht et al. (1997), the microvibrissae might be crucial for object recognition, while the macrovibrissae mostly play a role in spatial orientation. Thus, the close apposition of rats during social interactions and stimulus touch with the microvibrissae might be a natural sequence of whisker use for the exploration of complex objects. This is largely ignored by most studies of texture discrimination (Carvell and Simons, 1990; von Heimendahl et al., 2007; Morita et al., 2011), which focus on the macrovibrissae, which might be less sensitive to fine differences than the microvibrissae.

Close head apposition might, in addition, be a strategy to increase access to olfactory information, in particular pheromones from the cheek glands (Kannan and Archunan, 2001; Kiyokawa et al., 2004). That this is the sole reason for social facial touch seems very improbable, however. This is indicated by the stereotyped position that rats assume during interactions (Wolfe et al., 2011). The finding that the rats were oriented along one line during social interactions suggests that somatosensory signals were sampled and behavior was optimized for this sampling. If olfactory sampling was the dominant motivation behind social touch, more lateralized approaches would be expected, which were possible even under the constraints of the gap paradigm.

The firing rate map showed a peak at the location of close nose contact. This activity peak corresponded to the peak in occupancy, and thus BC neurons were activated most at the relative positions which were preferred by rats behaviorally. This suggests that rats align facially such that they maximally activate their BCs.

After whisker trimming, activity in BC decreased, and it was shown before that whisker trimming disrupts facial alignment and decreases the time spent in interactions (Wolfe et al., 2011). The decrease in neuronal responses during facial touch after whisker trimming might thus be the cause for the decrease of interaction prevalence after trimming. These findings also support the view that touch is a more important motivation for facial interactions than smell, as the presence of whiskers should have been irrelevant to the sampling of olfactory information. It should, however, be noted that whisker trimming might result in unspecific stress responses, and thus decrease the time spent in interactions independently of any specific

sensory information stream.

The close contact that rats established during social touch might also be the cause for irregular and small-amplitude whisking during social interactions, as compared to obect and stuffed rat touch. This might be due to both passive effects (the resistance met when subject whiskers hit stimulus head and/or whiskers) and by active modification of the whisking strategy (Towal and Hartmann, 2006; O'Connor et al., 2010b). A framework for an adjustment of whisking strategy which optimizes sensory inputs has been proposed by Mitchinson et al. (2007).

While preferred relative locations of the stimulus rat head were symmetric, the response fields of BC neurons showed a lateralization in line with the contralateral cortical representation of touch. This asymmetry seems very weak, however. This might indicate that neurons had wide response fields, which might have even extended bilaterally. Activation of neurons by ipsilateral facial touch would have to be mediated by interhemispheric connections. Such connections between the BCs are known (Pidoux and Verley, 1979; Armstrong-James and George, 1988a), and whether the net effect they exert is excitatory or inhibitory (Shuler et al., 2001), it might contribute to a symmetry in response fields.

An alternative, although not mutually exclusive explanation for the weak lateralization of response fields would be the spatial arrangement of stimulus features at certain relative positions. When the rat noses touched, the stimulus rat whiskers could already extend far into the whisker array of the subject rat. Thus, a contralateral whisker could even have been touched when the nose of the stimulus rat was still ipsilateral to the hemisphere from which signals were recorded. It is important to remember here that the occupancy indicates the relative position of the noses, not the distance of any available stimulus feature.

### 4.3.7 Oscillations in spike trains

Oscillations in spike trains were observed for different frequencies, and in some cases these oscillations were very prominent, in particular for frequencies beween 8 and 18 Hz. The diversity of these oscillations suggests that they were not, or at least not all, directly coupled to whisking cycles. At the same time, some units showed a high oscillatory activity immediately before interactions, which then decreased, and this seems related to the decrease in whisking amplitude and regularity with decreasing nose distance during interactions. It is also reminiscent of the pre-touch activity reported by Krupa et al. (2004) for infragranular layers, although the authors did not report oscillations. Taken together, it seems probable that at least some of the units were actually encoding whisker position. This would be expected based on the work of Fee et al. (1997), who report significant entrainment of 43% of BC SUs to whisker position. The authors found that the entrainment of 10% of cells was even sufficient to predict the position of the whisker, with the most represented whisker position being around maximal retraction and start of protraction. The example unit shown by Fee et al. (1997) to exhibit whisking-related oscillations was also a L5 cell, in line with the presented finding of strongest oscillations shown by L5 cells. Unfortunately, the authors did

not distinguish cell types and did not separate units by layers on the population level. Curtis and Kleinfeld (2009) also showed modulation of most units during active whisking, although these were found to be relatively weak.

There are other reports of oscillations in BC in the literature, where the relation to function is not clear. For example, Derdikman et al. (2003) using voltage-sensitive dye imaging observed an early touch-related excitation, a subsequent inhibition, and finally a late depolarization accompanied by 16 Hz oscillations. The function of these oscillations, which presumably stem predominantly from supragranular layers, and their relation to reports using other recording methods, is not clear. In addition, these data seem to contradict those reported by Poulet and Petersen (2008) for L2/3 neurons. These authors did not find correlations between the firing of L2/3 neurons during active whisking in mice, although it should be noted that oscillations in the firing of single cells can occur without a correlation between cells, as long as the cells oscillate at different frequencies.

Intrinsic oscillations in the 5-12 Hz range have been reported by Silva et al. (1991) in slices from L5 of rat sensorimotor cortex, but not in other layers. This is in line with the qualitative observation of strongest oscillations in L5. With regard to the exact pattern within L5, however, it should be noted that oscillations were observed to be particularly strong in L5B, while the paralemniscal inputs to L5A would make them most expected in L5A. Sobolewski et al. (2011) observed local field potential (LFP) oscillations in the same frequency range during quiet immobility. Although the relation of these oscillations to free whisking remained unclear, the finding that these were present in the absence of whisking and were decreased by tactile stimuli indicates that these are whisking-independent oscillations, which probably reflect a specific cortical attentional state. The study by Venkatraman and Carmena (2009) supports a relation to attentional state, as the authors found that microstimulation-evoked 15-18 Hz LFP oscillations in infragranular layers were strongest in quiet immobility. What is more, they observed a decrease in these oscillations as a function of whisking power, establishing a negative correlation between whisking strength and LFP power in this frequency range. The study of Derdikman et al. (2003) supports the view that these oscillations are not artifacts of microstimulation, but rather occur spontaneously in awake animals engaged in sensorimotor behaviors.

Oscillation frequencies below 12 Hz, which more closely resemble whisking frequencies, have also been reported by Semba and Komisaruk (1984). These authors found that whisking was strongly locked to either a 7 Hz theta rhythm originating from hippocampus or to a 9 Hz corticothalamic alpha rhythm. At the same time, it has been shown that during phases of cortical theta, the signal-to-noise ratio of supra- and infragranular BC neurons increases (Delacour et al., 1990), indicating that these are attentional states.

Overall, it seems that oscillations in the range of ca. 6-18 Hz both related and unrelated to whisker position are present in BC, and these might be stronger in the infragranular layers. Disentangling their functions might be difficult, because high attention is often accompanied by exploratory whisking. In this context, it would be interesting to score the behavior throughout the complete recording sessions and investigate oscillatory power in spike trains

for free whisking and quiet wakefulness independently.

**Oscillatory differences between RS and FS** On the population level, it was observed that over a wide range of ca. 11-27 Hz oscillations were much stronger in RS than FS. No data in the literature is available on oscillations in FS, and the functional significance of this finding remains unclear. It would be interesting to further investigate the oscillation patterns for different layers.

The quantification of oscillation strengths was relative to the overall oscillatory power in the frequency range analyzed, and thus it is important to exclude that the lower relative oscillatory power of FS at certain frequencies is an artifact of the overall higher firing rates of FS. However, this seems improbable, when the differences in RS and FS oscillations at frequencies above and below the 11-27 Hz range are taken into account. If a higher FS firing rate were the cause of the 'medium-frequency' differences, a compensatory dominance of FS oscillations at high frequencies would have to be expected. This could be an effect of normalization, as units firing at very high frequencies would have a higher power at high frequencies, and their normalized power at low frequencies would be automatically decreased in relation to that. This was not the case, however, and, what is more, FS showed stronger oscillations than RS in the lowest frequency range analyzed (3-8 Hz), which argues against an effect of firing rate on oscillatory strength.

### 4.3.8 Subject trimming

Whisker trimming experiments have been deployed widely to observe both behavioral changes (Thor and Ghiselli, 1975; Wolfe et al., 2011) and neuronal plasticity (Diamond et al., 1993; Lebedev et al., 2000; Margolis et al., 2012). It has been shown that in whisker-trimmed animals, social facial interactions decrease in number and the head alignment during interactions is disrupted (Wolfe et al., 2011). On the neuonal level, long-term plasticity induced by whisker trimming has been repeatedly shown to occur. In the paradigms typically used, one or two whiskers were spared, and responses to the stimulation of these whiskers were enhanced relative to the trimmed whiskers even after regrowth of the trimmed whiskers (Kossut et al., 1988; Glazewski et al., 1996; Margolis et al., 2012).

The trimming experiment reported here is different in that it was designed as a control and was not aimed at investigating plasticity and differential effects of whisker-trimming on neurons with RFs on trimmed vs untrimmed whiskers. It was also unusual in that whiskers were trimmed in the awake animal and neuronal responses were recorded immediately before and after trimming. This excludes long-term plasticity induced by trimming, and only short-term plasticity on the scale of $<$1h might have influenced the neuronal responses. There has been only one study previously which investigated the neuronal short-term effects of trimming in awake animals (Sellien and Ebner, 2007). The authors observed no decrease in the spontaneous firing rate of neurons, in contrast to the present data. A subtle but relevant difference between the two studies might be that in the whisker-trimming paradigm used by Sellien and Ebner (2007), whisker stumps of 3-4 mm remained, without which stimulation

of the 'trimmed' whiskers would not have been possible. In contrast, I cut the whiskers as close to the skin as was possible in the awake animal, which was ca. 1 mm. The pattern of response changes induced by their trimming protocol might thus not be directly comparable to the effects of 'complete' whisker-trimming reported here. Sellien and Ebner (2007) observed an initial 'reversed' plasticity immediately after trimming, where the stimulation of adjacent trimmed whiskers elicited stronger responses than the stimulation of adjacent untrimmed whiskers. This pattern was reversed 2h later and stayed so in further recordings. This seems to contradict the present study, where response magnitude was decreased immediately after trimming, but it is obvious that the changes of the principal whisker response induced by trimming of adjacent whiskers must not be similar to trimming of the principal whisker itself.

The finding that within the set of units recorded both before and after trimming, the only FS was also the only unit to slightly increase its activity, is in line with observations that excitatory but not inhibitory neurotransmission is decreased after sensory deprivation in S1 (Dolan and Cahusac, 1996). However, the long-term deprivation paradigm with its putative long-term plasticity effects, as used by these authors, is not directly comparable to the changes observed here on the time-scale of $<$1h, and the opposite effect has also been described for mice (Zhang et al., 2013).

### 4.3.9 Stimulus trimming

Most tactile stimulation during social touch seems to result from whisker-to-whisker contacts (Wolfe et al., 2011), and thus neuronal touch-related responses were hypothesized to be weaker when trimmed stimulus animals were touched. Unexpectedly, however, the stimulus trimming experiments showed no consistent differences in neuronal responses between the touch of trimmed and untrimmed stimulus animals. This observation might be explained by adaptive changes in the whisking strategy adopted by the subject rats when they interacted with trimmed stimulus rats. Possibly the subject rats brought their whiskers onto the snout of the stimulus animals, if stimulus whiskers were absent. This would be expected in the framework of the minimal-impingement strategy (Mitchinson et al., 2007), which predicts that rats adjust their whisking behavior to maximize the likelihood of tactile contact. This generates the hypothesis that whiskers were held more protracted and/or were moved at larger amplitudes during interactions with trimmed animals, not unlike the whisking patterns observed during object and stuffed rat touch. At the same time, it should be noted that such a compensatory strategy does not seem to preclude weaker responses during object than alive rat touch. Thus, the fact that the stimulus was an alive rat might have contributed to stimulus trimming-invariant responses independently from or in addition to a compensatory sensorimotor strategy.

### 4.3.10 Objects, stuffed rat and anesthetized rat as stimuli

The strong neuronal responses during social facial touch and their modulation by subject and stimulus sex (see 4.3.11) pose the question in how far social touch responses are different

from responses to the touch of other stimuli, which match some but not other properties of awake moving rats. This question can also be asked at the behavioral level: in what ways is the whisking behavior and, ultimately, the somatosensory input during touch of stimuli which are resembling awake rats in some aspects different to actual social touch? The three stimulus types considered in the present study were objects, a stuffed rat, and an anesthetized rat. Objects represent the control stimulus which is furthest removed from awake rats: they provide somatosensory stimulation, but their shapes are very different from rats. The stuffed rat was similar to the awake rats in shape, but it lacked the smell of awake rats, and was also much more stiff and had its whiskers more retracted. The anesthetized rat came presumably very close to the somatosensory qualities of awake rats. Importantly, it also had the smell of an alive rat, although it is unknown whether this might have been altered by anesthesia. Although it was immobile, its warmth, softness and breathing are cues which are presumably perceived by the subject rat during social interactions. The perceptibility of stimulus rat breathing is suggested, although not proven, by the observation of social transmission of food preferences (Galef, 1985), which acts through food-related olfactory cues present in rat breath (Munger et al., 2010). In contrast to awake rats, none of the stimuli was moving or did emit ultrasound vocalizations.

The responses of BC neurons during object touch indicated weakly but consistently lower firing rates than during touch of alive rats. The same trend was observed for the touch of a stuffed rat, but was less pronounced and remained overall inconclusive. Finally, the responses to the touch of anesthetized and awake rats were extremely similar. Although the number of neurons recorded during presentation of the anesthetized rat was low, a picture emerges in which the firing rates during touch of the different stimuli rank as follows: awake rat = anesthetized rat > stuffed rat > objects. This might indicate that BC firing rates scale with the importance of stimuli, and that firing rates are higher when stimuli elicit strong interest. This interpretation is in line with the observation of similar firing rates during the touch of intact and trimmed stimulus rats, although the sensory stimulation was presumably weaker during trimmed rat touch. Apparently, as long as some somatosensory input is present (which was not the case after subject trimming, where responses decreased), firing rates might reflect interest or relevance to a degree that masks effects from differing somatosensory inputs. In this context it is important to mention that the time spent in interactions with objects and the stuffed rat was quite low, while anesthetized rats were eagerly examined by the subject rat, as already observed by Blanchard et al. (1975).

It could not be answered in this study, whether the differences observed in firing rates in response to the touch of different stimuli were a function of differential whisking behavior. There are, however, indications that this plays a role. Whisking during touch of objects and the stuffed rat was strongly different from awake rat touch, in that during whisking onto inanimate stimuli the whiskers were held more protracted and the amplitude of whisking was much higher. In contrast, whisker motion during the touch of alive rats was much less regular and the set angle was less protracted. This is reminiscent of the work by Carvell and Simons (1995), who showed that the whisking strategy is adjusted to the respective stimulus object.

The parameters reported by the authors to be modified included set angle and whisking power, which were both found to be different between awake rat and inanimate stimulus touch in the present study. Although response indices during social touch and whisking parameters in single interactions were found to be largely uncorrelated, it is still to be expected that such clear differences in active touch behavior translate into neuronal response differences. The behavioral adjustments during interactions with inanimate stimuli might in fact have contributed to mitigate the effect of the behavioral relevance of different stimuli, in that for immobile stimuli whisking power was enhanced to optimize the strength of sensory inputs, in line with the minimum-impingement hypothesis (Mitchinson et al., 2007) discussed before.

It is interesting to note that the only significant correlation found between social touch responses and whisking parameters was a negative correlation between the whisking power of subject males, and their neuronal responses. Thus, responses were weaker for those interactions, which had higher whisking power and were therefore more similar to interactions with inanimate stimuli. It is possible that high whisking power was characteristic of interactions with weaker, immobile stimuli and/or large head-to-stimulus distances, and such interactions would also be expected to cause weaker responses. This interpretation would suggest a causal relation between response strength and whisking power, where weak responses cause large whisking power as a coping strategy with weak sensory inputs.

### 4.3.11 Sex-specificity of social touch responses

The firing rates and response modulations during social touch showed unexpected and partly very prominent sex-specific differences. The most striking difference concerned the higher interaction-related reponse increases exhibited by RS recorded from males. Regular-spikers recorded in males showed an overall excitation which was 2.3 times as strong as the excitation found in RS recorded from females. As of now, the neuronal basis of this effect is not clear. There seem to be at least three not mutually exclusive explanations. First, whisking behavior during social interactions could be different in male and female rats. Some differences in whisker set angles of male and female rats during facial touch were found by Wolfe et al. (2011). They seem, however, to be too weak to account for the physiological differences described here. Critically, the presented analysis of the correlations of whisking measures and response indices (see 4.3.13) argues against this explanation. Second, BC networks and/or neuronal excitabilities could be different between males and females. The present study does not allow to assess this directly, and more studies of sex-specific differences in BC organization using both extracellular recordings and other methods would be needed. Until now, only very few studies have been performed which addressed questions related to sex-specificity in BC. While sex differences were shown to exist between the densities (Cardoso et al., 2006) and cellular properties (Urban-Ciecko and Mozrzymas, 2011) of certain interneurons, as well as in lateralization (Tobet et al., 1993) and certain forms of plasticity (Dachtler et al., 2012), all these differences seem very specific and not capable of producing large-scale changes. To my knowledge, no study has been performed which would have directly compared neuronal firing

rates - whether spontaneous or induced - in awake males and females, in any brain area. Future studies might clear up, whether any as yet unreported substantial differences between male and female BC exist. Finally, the distribution of recorded SUs over layers differed considerably between male and female rats (Fig. 3.6). As already discussed in 4.1.4, layers could not be assigned to 40% of SUs, and thus the distribution in the dataset with determined layer provenience cannot be equated with the whole neuronal population recorded. Nevertheless, the much higher proportion of L5, in particular L5B, neurons in males than females, in conjunction with the observation of strongest response modulations in L5B, might indicate that some of the observed effects are due to imbalances in the origin of recorded cells in males and females. Importantly, there is evidence which argues against this explanation, and for the interpretation that the sex-specificity described here was not an artifact of differing recording locations. Thus, as described in 3.3.12, a comparison of L5B RS recorded from males and females showed that even for units from the same layer, neuronal responses recorded in male rats were stronger. Although this does not rule out a layer-imbalance effect completely, it supports at the very least a contribution of truly sex-specific differences to the observed effect.

A further sex-specific effect was the mean inhibition of female RS, when the female was in estrus and interacted with other females, but not males. This interaction effect of subject estrus state and stimulus sex is even more difficult to explain. Although this finding is statistically robust, it should be noted that it is caused predominantly by the absence of interaction-related spikes in some very low-firing units ($<$0.5 Hz). Whether this effect is generalizable to the whole RS population or is only typical of low-firing units, remains unclear. This finding should also be interpreted with caution, as for each unit each stimulus animal contributed one data point. Therefore, there are much more entries than units, and the entries can be seen as not completely independent. While this is a general concern in the analysis by single stimulus animals, it is much more relevant for a relatively small dataset, as was the case for responses of RS from estrus females (n = 32). Thus, it would be important to reproduce this finding in a larger group of neurons. In addition, it was not investigated, whether whisking differences could account for this inhibitory effect. Whisking differences seemed not to underlie other differential response modulations (see 4.3.13), and overall inhibition of BC neurons on the population level due to any kind of touch strategy would be new. Nevertheless, it could be investigated whether behavioral differences might contribute to this particular effect.

Taken together, two sex-specific differences were observed. First, male RS responses were stronger that those of female cells. This seems a robust finding, but its neuronal and/or behavioral basis remains unknown. Second, the RS recorded from females in estrus, uniquely, showed inhibition during social interactions with other females. Although this was a statistically robust effect as well, further investigations are needed to confirm and possibly qualify this finding. These sex-specific effects (also see 4.3.12 for the effect of estrus on firing rates) were unexpected and their magnitude exceeds the effect size of attentional modulation described before (Treue and Maunsell, 1996; Connor et al., 1997; Treue and Martínez Trujillo, 1999). It seems likely, although not certain, that the response changes observed here are qualitatively different from the BC properties predominantly studied so far. The effects observed seem not

well explained by sensory analysis of mechanical properties. Instead, it can be speculated that they result from attentional effects and/or multisensory integration. The mentioned studies on attention have been performed in monkey visual cortex, and it is unclear in how far they translate to rat somatosensory cortex. Attentional effects in BC have hardly been studied, and to my knowledge no study has addressed whisker-related attention with single-cell resolution. Similarly, there is only little data in the literature on multisensory integration. A recent study by Sieben et al. (2013) showed influences of visual stimuli on somatosensory responses in BC, but how differences in LFP power relate to neuronal firing rates, is unclear. The only quantification of cross-modal influences on BC SU firing rates comes from Ghoshal et al. (2011), who showed that firing rates in BC can increase by over 30% when touch is paired with a sound in a conditioning paradigm. Thus, multisensory integration of somatosensory with auditory and/or olfactory signals might contribute to the effects observed in the present study. At the same time, the existence of differences between males and females on the level of multisensory integration remains speculative, and how differences in auditory and/or olfactory stimulation could translate into such pronounced sex-specific changes in BC is unclear.

#### 4.3.12 Estrus effects on firing rates

Considerable differences were observed between the firing rates of BC neurons of female rats in estrus and non-estrus (Fig. 3.58), with RS firing at over 1.5 times higher rates in non-estrus. This was surprising, although neural changes over the course of the estrus cycle of female rats are well established. This is in particular true of the hippocampus, where it has been shown that spine density in area CA1 varies by 30% between different stages of the estrus cycle (Woolley et al., 1990), and that this has an effect on long-term potentiation and long-term depression (Good et al., 1999), as well as, ultimately, on neuronal firing rate in CA1, which decreases in proestrus (Tropp et al., 2005). Neuronal firing rates as a function of the estrus cycle have been compared for several other brain areas, including the lateral septum (Contreras et al., 2000), the ventrolateral medulla (Kaba et al., 1988), the striatum (Garcia-Munoz et al., 1989), and the olfactory bulb (Guevara-Guzman et al., 1997). However, to my knowledge, this is the first study which compared firing rates in any neocortical area as a function of estrus phase.

The observations from different brain areas indicate that there cannot be a simple correlation of estrus phase and neuronal firing in the whole brain. While Contreras et al. (2000) and Guevara-Guzman et al. (1997) observed higher firing rates in proestrus and estrus, Garcia-Munoz et al. (1989) found that firing in diestrus was higher than in estrus or proestrus, the phases with highest estradiol levels, and Kaba et al. (1988) found no differences in firing rates between estrus phases. The comparison of firing rates by estrus was not a main focus of this study, and thus, conclusions should be drawn carefully. In particular, and in contrast to most of the aforementioned studies, the minimal time that the estrus cycles were followed before the start of recordings was only two days and estrus cycle monitoring was interrupted in some cases.

Nevertheless, the observed differences in evoked and baseline firing rates between estrus and non-estrus indicate that the activity of BC is influenced by the estrus phase. It is unclear, whether this takes place through changes in excitability and/or through behavioral changes (Burke and Broadhurst, 1966; Erskine, 1989). The lack of comparable studies is to be seen in the broader context of a strong bias to use male animals in neuroscientific experiments (Beery and Zucker, 2011)[10], which comes about in part exactly because estrus-related neuronal and behavioral changes are to be excluded for the sake of standardization. But undoubtedly this is an important topic in itself, and as more recordings from female rats will presumably be performed in the future, it would be recommendable to determine the estrus phase and analyze any dataset along these lines as well.

#### 4.3.13 Weak correlations between whisking parameters and response indices

To investigate whether the sex-specificity of BC firing rates described in 3.3.12 was due to differential whisking behavior, whisking power and whisker set angle during single interactions were correlated with response indices. It was observed that correlations were mostly very weak and insignificant for both example units and a population of 52 RS, for which whisker tracking of corresponding interactions was available. This argues against differential whisking behavior as a major cause of the sex-specific response differences, including the stronger modulation of RS recorded from males, and the inhibitory responses observed in female-female interactions while the subject female was in estrus. The lack of strong correlations is in disagreement with the data reported by Fee et al. (1997), who found whisking envelope (i.e., the amplitude over several whisking cycles) to be correlated with responses for some units, although their findings refer to free whisking, and not phases of touch.

Of all correlations between whisking parameters and response indices, only the negative correlation between male response indices and subject whisking power was found to be significant. This small effect, which could only account for ca. 2% of the variation in the data, might be explained by small differences between interactions. This interpretation posits that interactions with larger head-to-head distances elicited weaker tactile responses, and that, at the same time, these larger distances led to a compensatory whisking strategy with larger whisking amplitudes. If the stimulus held its whiskers still, this might have had the same effect. However, a similarly negative correlation would then be expected between response indices and set angles, as set angles would be higher for larger head-to-head distances, but this was not the case. In addition, a negative correlation between subject whisking power and response indices was nearly absent for female cells. This also seems to argue against the above interpretation, although it could also be construed as an effect of different response properties between male and female neurons. Thus, it remains unclear whether the correlation observed in males describes a true effect, and if so, what the behavioral or neuronal basis of this effect

[10] Interestingly, as Beery and Zucker (2011) show, from ten biological fields, neuroscience has the strongest bias for conducting animal studies exclusively on males. What is more, this trend has increased for neuroscientific animal experiments between 1959 and 2009, while in human neuroscientific studies it decreased during the same period.

might be.

There are limitations to this way of correlational analysis, however, which leave the possibility open that subtle differences in whisking behavior were missed: First, the correlation of behavior and physiology took place on the level of single interactions. As the interactions with available high-speed tracking were on average ca. 1.5 s long, they can be expected to comprise 10-20 whisking cycles on average. Within these cycles, the whisking power and set angle might have changed, and in fact it was observed that the set angle decreased within interactions. If such changes were reflected by neuronal responses, this would not have been captured by the indices. In that regard, it would be conceivable to correlate neuronal responses to the amplitudes and set angles of single whisking cycles. Second, the exact touch events are not known, and the analysis is based on the assumption that the probability of touch events and their strength are independent of the sex of subject and stimulus animal. As female whiskers are presumably shorter and thinner than males', this assumption might be a simplification. Third, the assumption also enters that whisking is symmetrical. Preferably, the whiskers contralateral to the subject rat's implant were tracked, but this was not always the case, and although the rat midlines were aligned in most interactions, there were also strongly asymmetrical ones. While free whisking is typically symmetrical, whisker motion during object exploration has been shown to be asymmetric for asymmetric stimuli (Towal and Hartmann, 2006; Mitchinson et al., 2007). Thus, the side of whisker tracking might have influenced the observed whisking parameters. Fourth, while whisking of both rats during single interactions was typical, sometimes one of the two rats was keeping them still. This must not necessarily be associated with a decrease in responses, as passive touch responses in BC can be even stronger than those during active touch (Hentschke et al., 2006). Possibly a switch of modes between 'generative' and 'receptive', as proposed by Diamond and Arabzadeh (2013), accounts for strong responses with both actively moved and completely still whiskers. In any case and regardless of the effect that holding the whiskers still has onto firing rates, these two behavioral and possibly also neural states complicate the interpretation of responses, even more so, as they could potentially even occur within one and the same interaction. Finally, it should be noted that the pooling of responses over complete interactions disregards any possible temporal coding schemes. For example, Curtis and Kleinfeld (2009) observed whisking-related modulations, but these were not reflected in the overall firing rates of neurons, which remained unchanged. Thus, information on the strength of whisking might be present, but below the considered temporal scale. This last concern, however, does probably not apply to the interpretation of the sex-specificity of responses, as these were analyzed on the same timescale and using the same indices for males and females.

In sum, there is no simple correlation between whisking parameters and response strength at the timescale of single interactions. It remains to be tested whether an analysis on a finer timescale would produce a correlation. It should also be noted that whisking parameters are not the only behavioral parameters which could influence response strength during social facial touch, with facial proximity during interactions being one further important factor. Finally, increasing the number of tracked interactions would improve the analysis, and permit

the testing of e.g. estrus-related whisking differences. Nevertheless, the number of 865 combinations of unit and interaction seems already sufficient to tease out any relevant correlation of the investigated parameters with neuronal responses, and thus the presented data suggest that whisking behavior cannot or at least cannot completely account for the sex-specificity in BC activity on the timescale of social interactions.

### 4.3.14 Behavioral significance of social touch

Social facial touch is, as already shown by Wolfe et al. (2011), a behavior that rats show spontaneously and repeatedly. Wolfe et al. (2011) also observed a rebound effect, where rats who could see, smell, and hear, but not touch each other, would make up for this once given the chance. Nevertheless, it remains unproven that social facial touch is necessary or sufficient for any particular other behavior. While information on the stimulus animal might be present in both its shape and its whisking behavior, it is unclear, whether such subtle information is in any way used by rats. More behavioral studies on recognition and dominance will be needed to clear up possible roles of social facial touch on the behavioral level. Importantly, these studies will have to take into account the possibility that facial touch occurs partly or exclusively to sample non-volatile compounds from the cheek glands (Kannan and Archunan, 2001; Kiyokawa et al., 2004). These have been shown to be attractive to rats (Kannan and Archunan, 2001) and to increase the level of activity (Kiyokawa et al., 2004).

The present study characterized the neuronal responses to social touch, and showed strong social touch-related responses in BC, some of which probably went beyond the representation of purely somatosensory stimulus aspects. Nevertheless, the presence of such information in BC does not prove that this information is necessary for any particular behavior or decision, or in fact that this information is used at all. Once the behavioral relevance of social touch has been established more firmly, causal studies using optogenetic or other manipulations of BC will be necessary to clear up the flow of information from BC to upstream areas and its potential usage.

## 4.4 Outlook

There are three levels on which this study could inform further studies, apart from its immediate results and their aforementioned implications for sensory neuroscience. First, there are methodological considerations. Possible small improvements have already been mentioned before in the respective sections, but there is also potential for shifts to other recording techniques and experimental paradigms. Second, the gathered data could be analyzed in new ways and with different questions. Third, the presented findings could be a basis for further experiments in new directions.

**Methodology** There are several dimensions, along which the recordings could be improved as compared to the applied methods, and some could be considered for further similar experiments. Thus, the rapid development of neurophysiological technology has produced tools

which can lead to improvements with regard to (i) quality, (ii) quantity (i.e., unit yield), (iii) stability, and (iv) behavioral flexibility, although none of them can deliver all at the same time.

As shown in 3.1.3, recording stability decreases when tetrodes are relocated immediately before an experiment. Thus, as a low-level improvement to recording stability, the strategy of tetrode placement could be modified, such that tetrodes are never moved directly before an experiment. This would lower the number of recorded units, however.

Another strategy would be to keep tetrodes in the same location over subsequent days to record from the same units. This would be particularly interesting to validate the sex-specificity and estrus-dependence of responses on the level of individual SUs. Recordings from the same units over subsequent days using multi-wire electrodes similar to the ones combined to tetrodes in the present study have been reported to remain stable of several days (Ciocchi et al., 2010). Although tracking of cell identity within recordings over subsequent days is not a standard method currently for tetrode recordings, it can be assumed that the additional information available from tetrode recordings would lead to a further improvement of the stability measures developed for recordings with multi-electrode arrays (Dickey et al., 2009).

One speculative possibility for improvement of recording quality is the lowering of tetrode impedance by electroplating with additives, as described by Ferguson et al. (2009). By adding polyethylene glycol or multi-walled carbon nanotubes to a regular gold-plating solution, the authors reached impedances of 30-70 k$\Omega$ without producing short circuits, which could enhance the signal-to-noise ratio.

While the present study used extracellular signals, a more detailed picture on how complex social stimuli influence neuronal activity in BC could be gained by intracellular recordings. This could also give access to the information on neuronal up and down states present in cellular membrane potentials, and allow to quantify the interplay of these states with social stimuli.

Once an understanding of how social stimuli modulate neuronal activity has been gained on the level of neuronal layers and subtypes, this could be subject to causal interventions, as afforded by the optetrode (Anikeeva et al., 2012), which allows both extracellular recordings and the optogenetic manipulation of a specific cell type. Optogenetic techniques were originally developed in mice (Boyden et al., 2005), but are increasingly used in rats (Znamenskiy and Zador, 2013).

As described before, L1 activity is particularly difficult to record with tetrodes. At the same time, L1 could be a place where attentional or other context-related information enters BC. This would be one possible target for calcium imaging studies, which would also be valuable in observing plasticity and long-term changes in social touch-related neuronal activity. As for channelrhodopsins, there is the possibility of bulk-loading with calcium indicators, and although rats with genetically encoded calcium indicators are currently unavailable, it can be assumed that this will change. The miniaturized head-mounted 2-photon microscopes necessary for monitoring freely moving animals have already been developed (Helmchen et

al., 2001), and further impoved since. Calcium imaging of deep cortical layers also becomes increasingly available, although deep layers have been as yet only imaged in the mouse (Andermann et al., 2013). Overall, there is huge potential in the combination of these techniques and their long-term deployment to identify the circuits responsible for responses to complex social and non-social stimuli, similar to texture discrimination and object location as reported by Chen et al. (2013). Specifically, the opportunity to record activity of the same neurons over weeks and even months creates new ways to gather comparative data on response properties of cells with high statistical power, and to observe plasticity as stimulus animals become known or estrus states change.

Finally, in addition to improving electrophysiological recording methods, gathering of data on whisker movement could be made possible for all interactions and even over the course of complete experiments, if electromyographic recordings in the mystacial pad were used as a proxy of whisker position. This is a standard method widely used in the literature (Sachdev et al., 2003; Ranade et al., 2013), and the imprecision that it introduces with regard to the exact position of whiskers seems to be largely irrelevant for studies of social whisking, where the times of exact touch events are unknown in any case.

**Analysis** The presented analyses were largely restricted to interaction periods, as compared to a pooled baseline period. However, these periods in themselves include different behaviors, which might be associated with specific neuronal activity patterns. These different behaviors might in itself be interesting, while excluding them to obtain the times of quiet wakefulness for baseline calculations would further improve the presented analyses. Similarly, there were only two contact states defined between rats: 'in contact' and 'out of contact'. It would, however, be possible that the neuronal activity, in particular in the form of LFPs, is modulated by the proximity of a stimulus rat. Thus, a continuous tracking of the low-speed videos to obtain the positions of the two rats with concurring behavioral scoring would produce a new set of data which could be used to address questions related to attentional modulation, as well as the behavioral correlates of the observed oscillations in spike trains. To be feasible, this tracking would have to be automatized, and techniques which might deliver this have been developed. Amongst these, the one described by Matsumoto et al. (2013) is the most directly applicable for interacting animals, although adjustments might have to be made to deal with the tether cable and other idiosyncrasies of the chooser paradigm setting.

Due to the time consumption of the largely manual whisker tracking methods used here, whiskers were tracked for only a subset of interactions. The 230 whisker-tracked interactions correspond to 6.5% of the 3571 interactions observed overall. Although not all interactions can be tracked continuously due to whisker occlusion in parts of the interaction, there is a large amount of potentially trackable interactions, which could be accessed if whisker-tracking were possible in a more automatized fashion. Automatized whisker-tracking algorithms have been developed by several groups, and in particular the automatization achieved by the group of Karel Svoboda (Clack et al., 2012) is impressive. However, most algorithms are optimized for animals with only one row of whiskers remaining, and are not adjusted to deal with overlapping

whiskers from two rats, as is the case in social interactions. Thus, adjustments to these or other codes not yet applied to whisker tracking will be necessary to achieve considerable advances in automatization.

As LFPs have been acquired alongside spike recordings in most experiments, it would be possible to analyze whether differences in response patterns during interactions are associated with specific patterns of oscillatory activity, and whether this activity might even precede the spike responses. Combined with knowledge about the layer and cell type, this could generate testable hypotheses with regard to the mechanisms of social touch-related modulation.

The LFPs could also carry multisensory information, as has been described for social information in monkey auditory cortex (Ghazanfar et al., 2005). In particular social auditory information transmitted through ultrasound vocalizations might influence neuronal activity in somatosensory cortex. A modulatory effect of auditory stimuli on BC touch-related spiking responses was described by Ghoshal et al. (2011). Thus, it would be worthwhile to investigate whether such modulations can also be found in response to ultrasound vocalizations in both LFPs and spikes from BC. In fact, a possible relation of vocalizations and BC spiking responses was investigated for a small subset of neurons. No auditory responses were observed, but the study of Ghoshal et al. (2011) suggests that these cross-modal effects might be only present as modulations of responses to stimuli in the 'native' modality of a sensory cortex, and not by themselves. Designing the analysis accordingly, and with additional call detection and classification, the question regarding cross-modal integration of social signals in BC could be addressed in a considerable subset of $>$100 neurons.

Finally, taking a different direction, it would be possible to investigate whether the 600 Hz bursts observed in human (Curio et al., 1997) and monkey (Baker et al., 2003) somatosensory cortex are also present in rats, and if so, which stimuli and/or behaviors they are associated with.

**Questions** Although the sex-specific responses described here could not be attributed to differential sensory inputs or motor strategies, this explanation cannot be ruled out completely. In this regard, it would be interesting to record in the somatosensory nuclei of the thalamus to investigate, whether differential responses are already present at this early level.

The development of wireless multi-channel recording systems allows completely new experimental designs, such that a new methodology and a new experimental paradigm are linked. Using a wireless recording system as described by Szuts et al. (2011) would allow to observe how neurons respond to object and social touch in even more naturalistic settings, and for both facial touch and anogenital sniffing. In addition, such recordings could increase the number of interactions relatable to the activity of a given neuron, and thus improve the power to detect sex-specific or other modulations in neuronal activity.

Mice are widely used as models of neurological disorders, and mouse models of psychiatric disorders are also available. However, for psychiatric disorders which are mostly manifest in social behavior, in particular autism spectrum disorders, rats can potentially be better models. This is due to their larger size, which facilitates tracking and freely-moving electro-

physiological recordings, and their more docile behavior, which allows experiments in partly open environments as the gap paradigm, easier estrus measurements, etc. Arguably, rats also exhibit a larger diversity of social behaviors. To the point of this study, social facial touch is not exhibited by mice in the same fashion, and mice display barbering of subordinate male mouse whiskers by dominant males (Long, 1972)[11]. Thus, in the long run, rat facial touch and social whisking behavior might become one parameter in assays of social behavior in rat models of autism. Such models are currently developmental, where borna virus infections (Pletnikov et al., 1999) or prenatal exposition to valproic acid (Schneider and Przewlocki, 2005) cause phenotypes which reproduce some of the traits associated with autism. As the effects of these interventions are largely unknown and they presumably affect different systems and pathways, models which target identified pathways associated with genetic variants of autism might bear more promise. Such models are currently only available in mice (Peça et al., 2011; Schmeisser et al., 2012), but with the current breakthroughs in the generation of knock-out rats (Kitada et al., 2007; Geurts et al., 2009), similar models in rats seem within reach. In fact, some commercial suppliers have started to market knock-out rats. Still, the use of rat social facial touch in the assessment of autism models and possible interventions would require more behavioral work, in particular in a colony setting, as well as a wider deployment of methodological improvements including wireless recordings, EMG recordings from the whisker pad, and automated position tracking.

## 4.5 Concluding remark

This study is to my knowledge the first attempt at understanding the function of primary sensory cortices in social contexts and their representation of stimuli with social relevance on the cellular level. Behavioral monitoring at different timescales and the recording of neuronal activity in a considerable population of single units from barrel cortex were the mainstays of this study. In addition, I gathered data on the estrus state of female rats. This multi-faceted approach allowed a wider view on the relation between behavior and physiology, and this, critically, in freely-moving animals which performed a complex and natural behavior. The near-naturalistic paradigm, which avoided any extrinsic motivations through rewards, and where the animals were free to interact as often as they wanted, for as long as they wanted, and with all whiskers present, is a distinctive feature of this study. At the same time, it had the consequence that experimental control was limited, and many factors made a characterization of sensory input on temporal and spatial scales common in sensory neuroscience impossible. I addressed this, among others, by a transformation of interactions into a space centered on the subject rat nose, but the main limitation remains that the precise sequence and timing of whisker touches during each interaction remains unknown.

On the physiological level, I have taken into consideration the differences which might

[11] It has been questioned, whether barbering really is an expression of dominance (Sarna et al., 2000), but whatever its cause, it is a behavior repeatedly reported in the literature (Sarna et al., 2000; Kalueff et al., 2006; Nicholson et al., 2009), and thus seems a normal constituent of mouse social behavior, at least in laboratory conditions.

arise in different layers and different cell types, as far as the methodology allowed. However, the assignments to layers and cell types are partly incomplete or simplifying, and other functionally relevant distinctions like the differences between barrels and septa could not be considered. The short overview of barrel cortex connectivity given in 1.3 serves to further highlight its complexity and the care that should be exerted at interpreting results from even seemingly large neuronal populations.

That said, the results still strongly support the view that neurons in barrel cortex - and, by extension, in primary sensory cortices in general - can show responses which extend beyond simple stimulus properties, as typically ascribed to them. As any study, it leaves many questions open, and in particular it is largely restricted to the correlational rather than the mechanistic level. Nevertheless, it suggests the study of complex social as well as non-social stimuli and the associated responses in primary sensory cortices as a field, which can yield new insights on sensory computation in real-world settings, and thus, ultimately, a better understanding of how the brain performs the complex tasks of real life, a question which we are just beginning to address.

# 5 Summary/Zusammenfassung

## 5.1 English

Rats use their stiff facial hairs (whiskers) for somatosensation, and the pathway from the whiskers to the primary somatosensory cortex (barrel cortex) is well known. Rats also show diverse social behaviors, one of which is touch of conspecifics with their whiskers. Nothing is known, however, about the representation of these social touch signals in the brain, and whether it differs from the touch of non-social stimuli. Thus, the present study aimed at characterizing the neuronal representation of social touch signals in barrel cortex and comparing them with other somatosensory stimulation.

Using extracellular single-cell recordings in freely-moving rats, I show that a large part of neurons in barrel cortex modulates their activity during social touch. Here, the reported fraction of 40% of neurons is a lower-bound estimate. Responses were typically excitatory, but there were also examples of inhibition. The pattern of firing rates during interactions differed between cortical layers, with layer 5B being both the most active and the most strongly modulated. Layer 2/3 neurons had very low firing rates even during social touch, confirming earlier reports from patch-clamp recordings employing non-social stimuli. Regular- and fast-spikers could be distinguished based on their spike shapes, with fast-spikers showing more reliable but less strongly modulated responses.

In behavioral experiments rats preferred interactions with alive conspecifics over inanimate stimuli. Whisking strategies also differed substantially. Thus, inanimate stimuli were whisked at with regular palpating movements from protracted set angles. In contrast, social interactions were marked by irregular, small-amplitude whisking. Neuronal responses were also different during touch of alive rats and inanimate stimuli. Thus, objects elicited slightly but consistently weaker responses than alive rats. There was also a trend towards weaker responses when touching a stuffed rat.

I observed sex-specific differences in neuronal responses. The most prominent one was stronger modulation by social touch in regular-spikers recorded from males. This difference could not be explained by behavioral measures as whisking power and set angle, possibly indicating a neural origin of this difference. Regular-spikers from females fired much more weakly when the female was in the estrus phase of the estrus cycle. Furthermore, female-female interactions were associated with neuronal inhibition, when the subject female was in estrus. The latter effect was observed in very low-firing neurons only.

In summary, this is the first study which investigated social signals in a primary sensory area of freely-moving animals at the cellular level. It suggests that representations in sensory cortices might be less stimulus-driven and more top-down modulated than previously thought.

## 5.2 Deutsch

Ratten verwenden ihre Schnurrhaare (Vibrissen) zur Wahrnehmung von Berührungen, und die Leitungsbahn von den Vibrissen zum primären somatosensorischen Areal (Barrel Cortex) ist gut untersucht. Ratten zeigen auch vielfältige soziale Verhaltensweisen, darunter Berührung von Artgenossen mit ihren Vibrissen. Es ist jedoch nichts darüber bekannt, wie diese sozialen Berührungssignale im Gehirn repräsentiert sind, und ob diese neuronale Aktivität sich von der Antwort auf nicht-soziale Stimuli unterscheidet. Deshalb hatte die vorliegende Studie zum Ziel, die neuronale Repräsentation von sozialen Berührungen im Barrel Cortex zu untersuchen und diese mit anderer somatosensorischer Stimulation zu vergleichen.

Unter Verwendung von extrazellulären Einzelzellableitungen in sich frei bewegenden Ratten habe ich gezeigt, dass die Aktivität einer großen Zahl von Neuronen im Barrel Cortex während sozialer Berührungen moduliert wird. Hierbei stellt der beobachtete Anteil von 40% der Neurone eine untere Grenze dar. Antworten waren üblicherweise erregend, doch es gab auch Beispiele für Hemmung. Die Feuerraten während sozialer Interaktionen unterschieden sich zwischen kortikalen Schichten. Dabei war Schicht 5B zugleich die aktivste und die am stärksten modulierte. Neurone in Schicht 2/3 hatten sogar während sozialer Berührungen sehr niedrige Feuerraten, was frühere Berichte von Patch-Clamp-Aufnahmen unter Verwendung nicht-sozialer Stimuli bestätigte. Regulär-feuernde und schnell-feuernde Neurone konnten an Hand ihrer Aktionspotential-Formen unterschieden werden, und schnell-feuernde Neurone zeigten zuverlässigere, aber weniger stark modulierte Antworten.

In Verhaltensexperimenten bevorzugten Ratten Interaktionen mit Artgenossen gegenüber unbelebten Stimuli. Die Berührungsstrategien unterschieden sich ebenfalls deutlich. So wurden unbelebte Stimuli mit regelmäßigen Bewegungen der Vibrissen abgetastet, und diese dabei weit vorgestreckt. Dagegen zeichneten sich soziale Interaktionen durch unregelmäßige Bewegungen mit kleiner Amplitude aus. Neuronale Antworten während der Berührung von Artgenossen und unbelebten Stimuli unterschieden sich ebenfalls. Objekte lösten leicht aber konsistent schwächere Antworten als lebende Ratten aus. Es gab auch eine Tendenz zu schwächeren Antworten während der Berührung einer ausgestopften Ratte.

Ich habe geschlechtsspezifische Unterschiede in den neuronalen Antworten beobachtet. Der ausgeprägteste war die stärkere Modulation von regulär-feuernden Zellen in Männchen. Dieser Unterschied konnte nicht mit dem Berührungsverhalten während sozialer Interaktionen erklärt werden, was möglicherweise auf eine neurale Grundlage für diese Differenz hindeutet. Zellen von Weibchen feuerten deutlich seltener, wenn das Weibchen in der Östrusphase des Östruszyklus war. Zudem waren Weibchen-Weibchen-Interaktionen gekennzeichnet durch neuronale Inhibition, wenn das implantierte Weibchen im Östrus war. Letzterer Effekt wurde nur bei sehr selten feuernden Zellen beobachtet.

Zusammenfassend ist dies die erste Studie, die soziale Signale in einem primären sensorischen Areal bei sich frei bewegenden Tieren auf zellulärer Ebene untersuchte. Sie legt nahe, dass die Repräsentationen sensorischer Hirnrinde weniger stimulusabhängig und stärker top-down-moduliert sein könnten, als zuvor angenommen.

# 6 Appendix: Recordings in the striatum

The striatum is a subcortical area located directly beneath BC, and in one case a tetrode was positioned so deeply that responses of striatal neurons (n = 4) were recorded. Strong response modulations during social interactions were observed in these units, in particular around the onset of head touch. Figure 6.1 shows an example striatal SU, whose firing rate increased from 0.6 Hz during baseline time to 3.6 Hz during interactions (P = 0.001, permutation test). This unit was classified as a RS by spike shape, and thus was presumably a medium spiny neuron. These constitute over 90% of striatal neurons, and their spike shapes from patch-clamp recordings (Kreitzer, 2009) are reminiscent of the units classified as RS in cortical recordings.

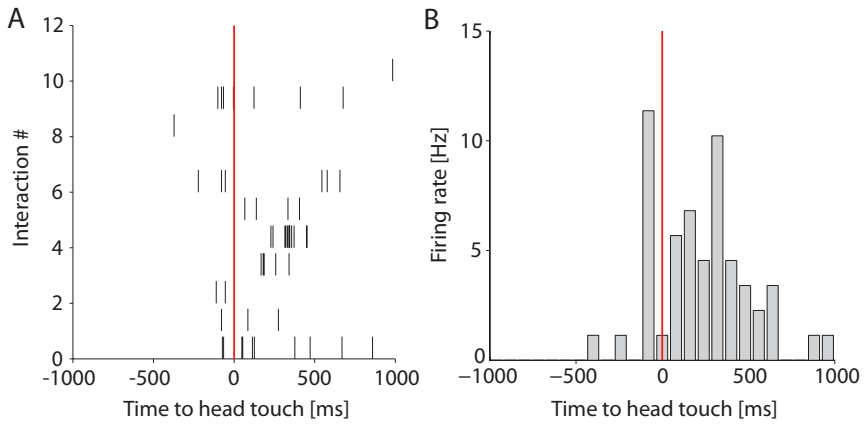



Figure 6.1: A, Raster plot of the responses of a striatal SU during social interactions, aligned to head touch. B, PSTH of the responses shown in A.

Clearly, the very small sample of striatal units does not allow for any quantification of striatal social touch responses. It is, however, an interesting observation which contributes to the view that the striatum is a very diverse area and plays many roles in addition to motor control (Albin et al., 1989). It has been shown that it contributes to decision-making (Znamenskiy and Zador, 2013) in rats, as well as working memory in humans (Voytek and Knight, 2010) and probably also rats (Kesner and Gilbert, 2006). Recently it was also observed that the dorso-lateral striatum of the rat encodes frequency of vibrotactile stimuli (Hawking and Gerdjikov, 2013). Although in the reported experiments touch-related and motor activity cannot be distinguished with certainty, the pattern of activity around interaction onset (Fig. 6.1), is clearly indicative of a touch-related response, as a motor-related activity would be expected to be stronger in the approach phase, when whisking is stronger and the body is moving towards the stimulus rat. In addition, no oscillations at whisking frequency were observed in any of the striatal units.

# 7 Abbreviations

| | |
|---|---|
| BC | barrel cortex |
| FS | fast-spiker |
| ISI | inter-spike interval |
| LFP | local field potential |
| M1 | primary motor cortex |
| MU | multi-unit |
| PSTH | peri-stimulus time histogram |
| RF | receptive field |
| RS | regular-spiker |
| S2 | secondary somatosensory cortex |
| SU | single unit |

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# 9 Publication list

### Research paper

**Bobrov, E**, Wolfe J, Rao RP, and Brecht M. (2013) The representation of social facial touch in rat barrel cortex. *Current Biology* 24(1):109-15. doi: 10.1016/j.cub.2013.11.049.

### Conference posters

**Bobrov, E**, Wolfe J, and Brecht, M (2012) "Barrel cortex responses to social facial touch in rats." Poster presented at Society for Neuroscience annual conference in New Orleans/USA (10/2012)

**Bobrov, E**, Wolfe J, and Brecht, M (2012) "Barrel cortex responses to social facial touch in rats." Poster presented at Barrels Society annual conference in New Orleans/USA (10/2012)

Rao RP, **Bobrov, E**, and Brecht M (2012) "Neuronal responses to conspecifics and social facial touch in the perirhinal cortex." Poster at the Society for Neuroscience annual conference in New Orleans/USA (10/2012)

Brecht M, **Bobrov, E**, and Lenschow, C (2012) "Cellular and synaptic analysis of the cortical representation of rodent social facial touch." Poster at the meeting of the German Scientific Foundation/Swiss National Science Foundation reasearch unit "Barrel Cortex Function" (10/2012)

Rao RP, **Bobrov, E**, and Brecht M (2012) "Activation of neurons in perirhinal cortex by conspecifics and social facial touch." Poster at the Federation of European Neuroscience Societies conference in Barcelona/Spain (7/2012)

**Bobrov, E**, Wolfe J, and Brecht, M (2011) "Barrel cortex responses to whisker touch during social encounters of rats." Poster presented at Society for Neuroscience annual conference in Washington, D.C./USA (11/2011)

**Bobrov, E**, Wolfe J, and Brecht, M (2011) "Barrel cortex responses to whisker touch during social encounters of rats." Poster presented at Barrels Society annual conference in Baltimore/USA (11/2011)